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VOL. V, No. 1 OCTOBER 1953

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

MARTINUS NIJHOFF — PUBLISHER — THE HAGUE

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol

Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten

Issued under the auspices of the "Nederlands Genootschap voor Landbouwwetenschap" (Netherlands Society of Agricultural Science)

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Printed in the Netherlands

Plant and Soil V. 1-100 The Hague October 1953

Plant and Soil V, no 1 October 1953

GROWTH AND ROOT DEVELOPMENT OF LOWLAND RICE AND ITS RELATION TO OXYGEN SUPPLY *)

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INTRODUCTION

Among the important grain crops of the world rice holds a very peculiar position. Although the methods of cultivation may diverge somewhat in the different countries they all have in common the fact that the rice fields are under water almost up to the time of harvest. There are rice varieties which can be grown successfully on dry land (upland rice), but it is stated (Jones [10]) that these varieties, too, give a better yield when grown as wet rice even when the upland conditions are optimal with regard to soil structure, moisture content and fertility. One is forced to conclude that the paddy field is of some advantage to the rice plant, an advantage which is not yet fully understood.

In Indonesia whole ears or kernels are laid out or sown on a nursery bed and the seedlings are transplanted, one to five or six in a hole, in the paddy field. Both the nursery bed and the field are carefully ploughed and harrowed with an ample supply of water (puddling) and the fields are waterlogged until the ripening stage of the plant. A shortage of water supply sometimes makes a deviation from this procedure necessary but the ordinary method is always preferred.

In these waterlogged fields heavy reduction takes place (Robinson [25]), Sturgis [30])). This occurs not only on very heavy soils or on soils with a high groundwater level but even in paddy fields on highly porous lateritic soils, often with a very low water

*) This work was carried out at the Institute for Botanical Research, General Agricultural Research Station, Bogor, Indonesia.

table. When the content of free iron and manganese of these soils is high enough a very hard concretion is formed just below the ploughpan, consisting of separate Mn- and Fe-layers. The Mn-layer especially is very hard and impenetrable to the roots of rice (Koenigs[14]). De Gee[4]) measured the oxidation potential in these soils and found that just below the Mn-concretion layer, it rises very sharply to values which equal those found in the irrigation water above; in between these horizons, very low values are found.

Many plants which grow with their roots in a reduced medium possess special means of securing the oxygen supply of these roots. Some of them, e.g. Nymphaea, contain large air channels in leafstalks, stem and roots. Others possess a special type of respiratory root extending into the upper layers of the mud and the water above, e.g. the pneumatophores of mangroves. Further, it has been shown that during winter, when they are not in contact with the air above, the rhizomes of Nuphar and other plants of the same habitat have an enzyme system which enables their tissues to respire anaerobically (Laing[20]). This form of adaptation to a medium without oxygen, however, is only a temporary one, as the products of fermentation accumulate in the tissues and at high concentrations may be harmful. This adaptation can only be helpful during a relatively short time or during dormancy when there is little demand for energy.

With lowland rice some research has been done on the oxygen content of the paddy fields and the oxygen supply of the roots. Harrison and Aiyer[6]) [7]) [8]) investigated the composition of gases escaping from paddy fields. These gases consist mainly of methane and nitrogen and contain practically no oxygen. According to the authors, however, this does not mean that the rice roots cannot obtain any oxygen from their surroundings. At the soil surface methane is utilized by bacteria and oxidized to carbon dioxide, which, in turn, is used by algae in this layer in the assimilation process, by which oxygen is liberated. As a consequence of transpiration oxygenated water permeates the soil down to the roots. It is even supposed that this soil solution moving downward is supersaturated with oxygen.

Several objections to this hypothesis can be made. Harrison and Aiyer have clearly demonstrated that oxygen is evolved at the soil surface, but there is no evidence as to how rapidly this oxygen is consumed again by organisms in the deeper soil layers. It

seems rather contradictory that methane and many other reduced substances (such as manganous and ferrous compounds, ammonia, and others) can be formed in the soil at places where there is a stream of oxygenated water moving downwards. Although many authors (see for instance Robinson[25]), Sturgis[30]), De Gee[4])) have demonstrated the severe reduction of paddy fields below the first two centimetres of soil, the views held by Harrison and Aiyer on the oxygen supply of rice roots are still accepted in some textbooks on the subject (Russell[26])).

On the other hand there are publications which indicate other means of oxygen supply. Sturgis[30]) mentions the incrustations of ferric compounds around the roots of rice and supposes that oxygen diffuses outward from the roots, oxidizing the ferrous compounds around the roots to insoluble ferric compounds. Vlamis and Davis[32]) [33]), too, criticize the hypothesis of Harrison and Aiyer and find that rice plants thrive well in culture solutions through which a stream of nitrogen is bubbling. Salt uptake is quite as high as in aerated controls. When the roots are excized, however, salt uptake in a medium containing no oxygen is seriously hampered, and the writers conclude that oxygen must of necessity pass downwards through the plant itself. This is confirmed by Lin[22]) working with rice in culture solutions bubbled with nitrogen.

That the rice plant can indeed supply the roots with oxygen in this way was clearly demonstrated by Van Raalte[23]) [24]) who analyzed the oxygen content in the air channels of the root cortex. The percentage of oxygen was the same whether the roots were in a normal or in a heavily reduced culture solution, but, in the latter case, the oxygen concentration dropped immediately when the shoots were cut off and the stumps sealed with paraffin wax. Further it could be shown that the roots of intact plants gave off oxygen to a reduced solution, thus confirming the view held by Sturgis.

From the literature cited it can be concluded that rice is able to grow in a soil containing hardly any oxygen. The oxygen necessary for the roots is transported through the plant itself by means of large air spaces mainly in leaf sheaths and roots.

Moreover, it appears from literature on this subject that an oxygen transport from shoot to root is not restricted to swamp plants. Cannon[3]) considered the possibility of such a transport by Helianthus and Salix; Leonard and Pinckard[21]) by cot-

ton. Glasstone [5]) examined several plants in experiments in which the medium around the roots was hermetically sealed from the medium around the shoot. She was able to demonstrate a gas flow through the plants examined. The rate of this flow was rather high; in the case of *Nicotiana silvestris* it amounted to 15 litres per hour, with a pressure of 1 cm butylphthalate.

Several authors have considered the possibility that it is predominately the oxygen liberated in photosynthesis which is transported to the roots. Cannon [3]) states that the oxygen consumption by the roots of Helianthus and Salix is greater in the dark than in the light and supposes that in the light oxygen is supplied through the shoot also. Laing [20]) showed that the oxygen pressure in the rhizomes of Nuphar was much lower in the dark than in the light and he supposes that oxygen liberated in photosynthesis is supplied to the rhizomes. Van Raalte [23]) was not able to find any difference in oxygen concentration in the roots of rice whether the shoot was in the light or in the dark.

Oxygen transport from shoot to root whether favoured by photosynthesis or not is, however, not the only adaptation to a reduced medium. Cannon [2]) (cited by Sturgis [30])) found that rice seeds were able to germinate at an oxygen concentration of 0.5 per cent. Taylor [31]) has reviewed much of the literature on this subject. The results of his own research are in accordance with the outcome of earlier experiments and with the result cited above. Unlike wheat, rice was able to germinate and to grow under very low oxygen concentrations. The carbon dioxide output under anaerobic conditions was 1.5 times as high as under normal conditions; for wheat this factor was 0.5. This suggests a superior fermentation system in the case of rice. Taylor was able to show that both wheat and rice can utilize ethyl alcohol in respiration but that rice is more tolerant of this principal end product of carbohydrate fermentation. Vlamis and Davis [32]) found that whereas rice seeds could germinate in oxygen-free water barley seeds could not, but contrary to the results of Taylor these authors did not find any difference in relative carbon dioxide output between barley and rice at low oxygen concentrations. Lin [22]) did not find much difference between rice and wheat seedlings in their ability to grow under low oxygen pressure. Contrary to the experiments of Taylor and Vlamis and Davis, his experiments were carried out in the light.

Of the three forms of adaptation to a reduced medium around the roots of plants mentioned earlier in this section it has been shown that rice possesses at least two. But there are indications in the literature on this subject that it also possesses the third, *i.e.* the existence of a specialized type of root serving to obtain oxygen from the surface and transport it to the roots below.

Harrison and Aiyer[6]) mention that in their pot experiments rice developed a more superficial root system in undrained pots than in drained ones. They suppose that in undrained pots the water oxygenated near the surface film can hardly penetrate the soil below. When there is excessive drainage, however, this surface film is not well developed and, consequently, the penetrating water is not sufficiently oxygenated to supply the needs of the root system. Growth therefore is rather poor in both cases. The best condition for the growth of rice is a soil with moderate drainage. In this way a well developed surface layer is established; water penetrates far enough and it contains enough oxygen to ensure an even development of the root system in the mud. Thus, according to Harrison and Aiyer, the root system of rice only develops in those parts of the soil where the solution contains enough oxygen to meet the needs of the plant. In a later paper [8]) the authors state that rice develops a superficial layer of very fine roots which prevents the escape of soil gases developed below it. When the rice plant is in the heading stage this layer degenerates and the gases escape to the surface.

Sethi[28]) also observed the development of a superficial layer of fine roots. He states: "These (laterals) were the finest in nature, and by intermingling with the surface algal growth formed a very delicate tissue of fibrous belt...." (p. 64). He supposes that these roots especially function in absorbing the oxygen dissolved in the irrigation water. During flowering the root system as a whole degenerates gradually. The development of this fibrous belt further depends on the condition of the soil and the rice variety employed.

Although several authors have connected this layer of fine, abundantly branched roots at the surface of the soil with the oxygen supply of the rice plant, no definite proof of such a connection has yet been given. There are two main possibilities. The first is that mentioned by Harrison and Aiyer, viz. that the roots only develop where the soil solution contains enough oxygen; in this case

the superficial layer has no function in transporting oxygen to other roots. The second is that the surface roots absorb the oxygen and transport it to places where none is available. The implication is that these roots are functionally different from other roots.

The work described in the following sections was undertaken in order to get a clearer insight into the oxygen supply of the roots of rice.

THE TRANSPORT OF OXYGEN FROM SHOOT TO ROOT

That the rice plant is indeed very tolerant of the oxygen content of the medium around the roots is demonstrated by the fact that it can be grown well in a nutrient solution without aeration. This can not be said of many other tropical plants.

K u i l m a n [16) 19)], successfully cultivated rice on a slightly modified Zinzadze-solution without aeration. He mentions, however, that a severe reduction may take place, as a result of which the roots are blackened. This reduction was apparently not at all harmful to the plant.

In the present experiments on Hoagland-solution a reduction often took place a little before the ears emerged. The solution and the roots were coloured deep black and gave off a strong smell of hydrogen sulfide. Contrary to the experience of K u i l m a n, however, it was observed that this reduction was distinctly harmful to the plant. As a consequence of individual variation, the reduction did not begin at the same time with all plants of the same series. At this developmental stage the plants in a reduced solution could be clearly distinguished from the others since more of their leaves died off. This did not apply, however, to the young leaves nor was the further development of the plant seriously hampered although the plants were less robust. At the end of the vegetative period the reduction of the solution usually diminished. Thus, although the harmful effect of the reduction was not very great, it could be clearly distinguished as such.

This reduction was never observed during earlier stages in the development of the rice plant. It only occurred a short time before the ears emerged and often there was no reduction at all during the whole vegetative period. There was some indication that it might occur more often during the dry monsoon than during the wet monsoon.

This reduction of the culture solution was the reason for a renewed investigation into the oxygen supply of the rice roots since it seemed possible that oxygen transport through the plant itself might not be sufficient at certain stages of development to cover the needs of the roots. In his experiments on transport of oxygen through the roots Van Raalte does not mention the age of the plants used, so that no conclusions as to this possibility can be drawn from his experiments.

That this possibility is a real one is clearly seen when one considers the development of the rice plant. A clear description of its development has been given by Kuilman [17) 18)] and Kerling [12) 13)] (with further literature on this subject). It appears that two main periods of development can easily be distinguished. The first is the period of tillering, the second that of stem elongation and ear development. Depending on the season and the variety employed, the lapse of time between the two periods differs. Stem elongation sometimes starts before the grand period of tillering has ended, but more often these two periods are separated by a longer or shorter interval of apparent inactivity. During the period of stem elongation the anatomical features of the plant are markedly altered. At first there is a very short cone-shaped stem with the successive leaf sheaths implanted upon it. As a consequence there is only a very short distance between the base of every leaf sheath and the proximal end of the roots (fig. 1*a*). As soon as elongation starts this distance increases with decreasing age of the leaf sheath as is shown diagrammatically in fig. 1*b*. In addition, during this period the oldest leaves die off so that soon after the beginning of the period of stem elongation there is a considerable distance between the base of the roots and the base of the oldest healthy leaf sheath.

As Van Raalte has shown, oxygen can be transported downwards through the plant to the roots. The leaf sheaths contain large airspaces and the cortex of the roots air channels. Before the elongation of the shoot the oxygen has only to overcome the resistance of the connective tissue between leaf sheath and root but as the stem elongates this resistance will become greater as the nodes develop and the distance increases (cf. Juliano and Aldama [11)]). Thus the question arises as to whether elongation of the stem initiates the reduction of the culture solution during heading time by hampering oxygen transport through the plant; and, if this is the case, how does the plant in the field overcome this difficulty.

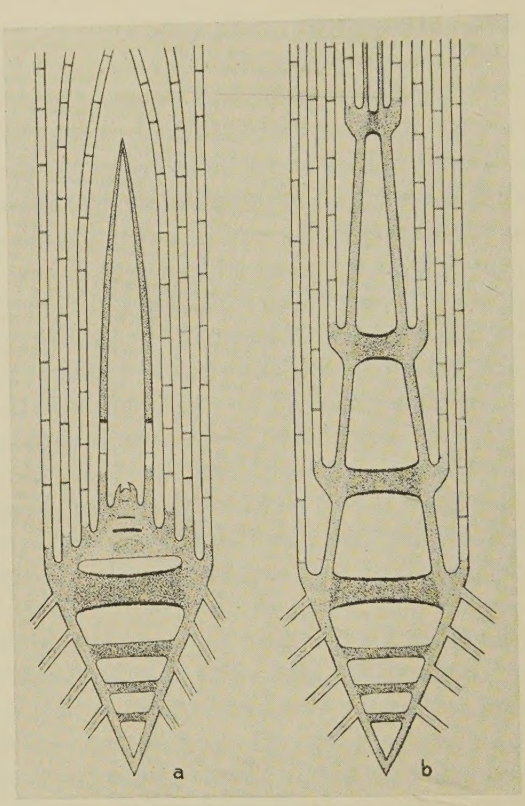

Fig. 1. Longitudinal section of a rice plant before (a) and after (b) stem elongation (diagrammatic).

Experimental work

In order to investigate whether oxygen transport through the plant is retarded after the elongation of the stem, experiments were carried out with rice cultivated in a nutrient solution.

Methods: Rice seeds of the Bengawan variety were sown in an earthenware pot filled with paddy soil mixed with a little horse manure. When the seedlings were about ten centimetres high they were transferred to the culture solution. Cylindrical vessels of one litre capacity, successively covered on the outside with a layer of black and a layer of white paint, were filled with a nutrient solution after Hoagland and Broyer[9]) with A–Z supply (cf. Schropp and Scharrer[27]) *). In each vessel one

*) As ferric tartrate was not available ferric citrate was used in a concentration of 5 cc per litre of a 1% stock solution.

seedling was fixed in the central hole of a wooden cover. The plants were able to tiller freely. When the roots reached the bottom of the vessel the plants were transferred to four litre cylinders. With a weekly renewal of the solution this method proved quite satisfactory for cultivating rice plants until the end of the vegetative period.

As already mentioned, a heavy reduction often took place in this solution at the end of the vegetative period, usually a little before the ears emerged. This reduction may partly be caused by bacteria in the culture solution. That the presence of an old plant was necessary, however, appears from the following considerations:

1. In a culture solution without a plant a reduction never occurred.
2. When the plant was removed from a reduced solution the reduction disappeared very quickly.
3. When the plant was removed from a reduced solution and a young plant was introduced instead no trace of a reduction was left after 24 hours.

The following experiment shows that, with an old plant, the oxygen consumption by the roots from the solution relative to the diffusion of oxygen from the roots into the solution is indeed greater than with a young plant.

In a culture vessel with a flowering rice plant severe reduction took place. The oxidation potential of the solution was measured at different depths with a Pt-electrode. After these measurements had been made, a young plant was introduced into the same vessel together with the old one. After three days the potential was remeasured at three different places; viz. between the wall of the vessel and the root system of the older plant; between the root system of the old and that of the young plant and between the young plant and the wall. These results are given in table I.

It is evident that the potential on the side of the young plant was higher than on the side of the old one throughout the whole depth of the culture solution, the greatest difference being 185 mV. Although the potential in a vessel with a plant and a reduced solution may vary considerably with the position of the electrode (different depths and the position relative to the root system) in the present experiment these differences were never so great as those between the potentials at the side of the young plant and at the side of the older one. In addition, the higher potential was constantly found on the side of the young plant. A repetition of this experiment gave virtual-

ly the same results. The increase in the oxidation potential below a depth of 20 cm was apparently caused by the fact that the roots did not all reach the bottom of the vessel.

TABLE I

Oxidation potential in a culture vessel containing a rice plant in heading stage before and after the introduction of a young rice plant in the same vessel. Introduction on 19-2-'51 just after the first heading.

depth	19-2-'51	22-2-'51		
	old plant only	old plant with young plant		
	oxidation pot.	oxidation potential		
		on side young plant	between old and young	on side old plant
cm	mV	mV	mV	mV
2	155	190	125	160
7	115	165	45	145
12	65	150	10	35
17	—25	150	—5	—35
22	?	150	95	80
27	85	150	100	90

Although the introduction of the young plant into the vessel increased the total mass of roots (and consequently the oxygen consumption) the oxidation potential in the solution rose considerably. This means that the young plant was not only able to obtain sufficient oxygen from the air but even to give some off to the surrounding reduced solution. The findings of Van Raalte that oxygen can diffuse through the outer cell layers of a rice root into a surrounding solution are thus confirmed.

However, this experiment did not demonstrate that with an old plant oxygen transport through the plant itself is hampered. It remains possible that the oxygen concentration in the root cortex is high and that there is no oxygen consumption from the surrounding solution. In that case the oxygen in the solution would be consumed by bacteria, an old plant being necessary for the reduction only as a supplier of decaying organic matter. That this is not so was demonstrated by the following experiment.

The oxygen content of the gas in the airchannels of the root cortex of young and old plants was determined with a Krogh-pipette, following the method given by Krogh [15]). The samples were taken according to the method described by Van Raalte [23]).

In table II some values are given of the oxygen content of the gas in the roots of a young plant in its tillering stage and of an old one that had already brought about a reduction of its culture solution. Since it was necessary to measure carbon dioxide concentrations, these values are also given.

TABLE II

Percentage carbon dioxide and oxygen in healthy roots of a young plant in a normal solution and of an old plant in a reduced solution.

young plant		old plant	
CO_2	O_2	CO_2	O_2
%	%	%	%
3.1	7.8	5.6	0.6
3.7	5.9	5.7	1.0
3.5	9.2	6.1	0.7
4.3	7.6	4.2	0.4
3.1	4.8	5.7	2.1
4.8	7.4	7.7	2.6
2.7	9.8	5.9	0.0
3.8	5.7	3.9	0.0
4.2	7.6	3.0	3.4
7.1	10.7	4.5	0.2
9.2	9.3	4.2	0.4
7.5	11.6	6.8	3.1
7.0	11.1	3.7	0.5

It appeared that the oxygen content of the gas in the roots of the old plant was very low indeed. Therefore it must be concluded that in the case of old plants the oxygen supply to the roots was very much retarded as compared with young ones. However, this was only the case when the solution was reduced. In plants of the same age but in a non reduced nutrient medium the oxygen content of the root gas did not differ significantly from that of a young plant. Therefore it cannot be said with certainty that the elongation of the stem can cause a retardation of the oxygen transport through the plant and, consequently, a reduction of the culture solution. This retardation can only be ascribed to some developmental factors, which operate under certain circumstances. These factors need further investigation.

THE DEVELOPMENT OF THE ROOT SYSTEM IN RELATION TO OXYGEN SUPPLY

Once it had been established that in the second half of the vege-

tative period oxygen transport through the plant is very often retarded, the question arose as to how the roots in the strongly reduced paddy soil are provided with oxygen.

As has already been stated, Harrison and Aiyer and Sethi noted the development of a superficial layer of roots in connection with the oxygen supply of the rice plant. The former workers believe that rice roots develop abundantly only in places where sufficient oxygen is available in the immediate vicinity. Sethi, on the other hand, considers it possible that oxygen is transported from the superficial roots to other roots situated at greater depths. However, these authors agree that the superficial roots degenerate after the beginning of stem elongation. If this is so the root mat could scarcely play a part in the oxygen supply of the whole root system in the latter half of the growth period.

However, the present writer has found in preliminary observations that a distinct root mat is present when the plants are heading, but that no such mat could be found on young rice plants. The first aim of the present work, therefore, was to extend these incidental observations.

1. *Observations on plants growing in the field*

Since Bogor is situated in a region of very high rainfall, it was possible to study rice plants in all stages of growth at the same time in the same area. It was, thus, possible to examine root systems of different varieties on different sites and at different times of the year. In this way the results were not restricted to a particular variety, season, or intensity of soil cultivation. The only limitation concerns the soil which was lateritic in all cases.

In order to obtain clear-cut results the growth period of the rice plant was divided into 13 distinct stages.

The material was harvested from the paddy field by hand, and the soil removed by washing in running water. The plants were then removed to the laboratory for further examination. The shoot was cut off and a vertical section made through the root system. These sections were examined in a shallow white enamelled dish, filled with water.

An objection to this method is that one is not able to reproduce the exact relative position of the roots in the field. However, three facts minimize this difficulty. The first is that the majority of the

roots forming the superficial mat grow in a horizontal direction from the base. From figure 2 it can be seen that the position of the basal part of these roots is more or less fixed by the vertical roots, so that it is difficult to alter their relative position.

Fig. 2. Root system of rice; coarser type of root growing downwards; finer type growing horizontally.

Secondly, the apical zone of the superficial roots is often covered with unicellular green algae and, consequently, these roots must have grown partly either in the irrigation water or on the surface of the soil. Finally, the superficial roots are generally thinner and more extensively branched than the others. Guided by these three features it was possible to ascertain whether or not a root mat was present and more elaborate methods were unnecessary.

In the course of this study it was possible to distinguish not only a special type of horizontally growing root but also roots of the same type growing vertically through the irrigation water into the air above. These roots grow in the centre of the stool and partly between the stem and the leaf sheath; thus they are protected from excessive drying.

From a large number of observations it could be seen that neither kind of root (horizontal or vertical) is present during the first half of the period of tillering. At the end of this period, however, many short white roots can be observed growing horizontally. During subsequent growth periods the two types of root gradually develop and they are always present when ear formation begins. They remain at least until the ripening stage. (At this stage it is usual to remove the

water from the paddy field and this practice precluded observations up to the time of harvest).

Thus it could be clearly seen that two types of specialized root are present during the second half of the vegetative period. Beginning with the period of stem elongation, there is a gradual development of a superficial root mat and no sign of degeneration of this mat has been observed in the present experiments.

2. *Observations on plants growing in pots*

Since part of the root system is unavoidably ruptured by digging it out by hand and, further, since it is difficult to reproduce, after washing, the exact position of the roots in the field, it was thought worth while to examine the roots of plants growing in pots.

Sixteen square unglazed earthenware pots ($20 \times 20 \times 20$ cm) were used; they were first coated with paraffin wax on the inside and then filled with dried, ground, and sieved paddy soil to about 5 cm below the rim. The soil was brought into puddling condition by kneading it with water. After the soil had settled the layer of water above the soil was removed and one young rice plant (Bengawan variety) planted per pot. As the plants grew, the level of the water over the soil was gradually raised to a height of about 3 cm.

At intervals the water in one of the pots was poured off and the soil allowed to dry for 24 hours. During this time the whole contents of the pot shrank somewhat and could easily be removed. A vertical section of about 3 cm thickness was then cut out of the block of soil through the centre of the plant. This section was laid upon a board bearing nails 2 cm apart. The soil was then carefully washed away with a small jet of water, the roots remaining almost exactly in position. During the course of the vegetative period seven plants were thus examined.

With regard to the time of development of the root mat the data of the field observations were fully confirmed. Five weeks after transplanting the first white horizontal roots could be seen in the irrigation water, but only in a few pots. Four weeks later these roots were observed in all sixteen pots. After a further four weeks all plants possessed a well developed root mat. Eight weeks after planting the grand period of tillering had just finished. In fig. 3 the full line represents the amount of tillers; the dotted line the number of pots which showed the beginning of root-mat formation; and the

broken line the number of pots which had a well established root mat already (total number by this time 14 since 2 pots had been covered with oil).

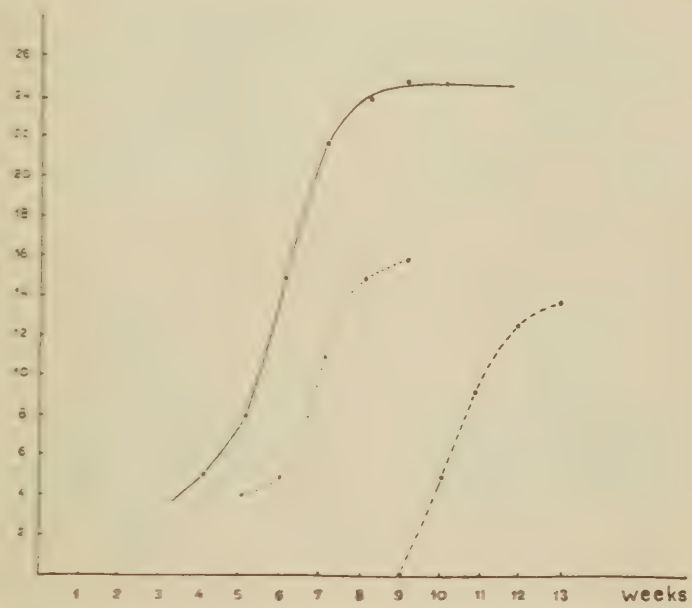

Fig. 3. Correlation between tillering and root mat development (see text).

From the sections made it could be seen that eight weeks after transplanting the roots were growing throughout the soil but a root mat was not yet present (see pl. 1). Three weeks later, however, there was a distinct concentration of roots at the surface. There was also a concentration of roots at the bottom of the pot but this was evidently caused by the fact that these roots could not continue downwards and turned horizontally. For the same reason there was a slight concentration of roots near the walls.

Though a root mat can be clearly distinguished about ten weeks after transplanting, it appears from pl. I that such a mat forms only a very small part of the whole root system. In every cubic centimetre of soil fine rootlets can be found.

De Geer[4] has clearly demonstrated that the oxidation potential falls rapidly in the upper few centimetres of soil. In order to test this in the present experiments the oxidation potential was measured

with a blank Pt-electrode. This electrode was mounted on a microscope tube and by turning the coarse screw one eighth of a full revolution after each measurement a continuous series of readings was obtained from the irrigation water downwards into the soil at successive distances of 2 mm. The results of four such series, measured on successive days, are given in fig. 4. It appears that the potential in

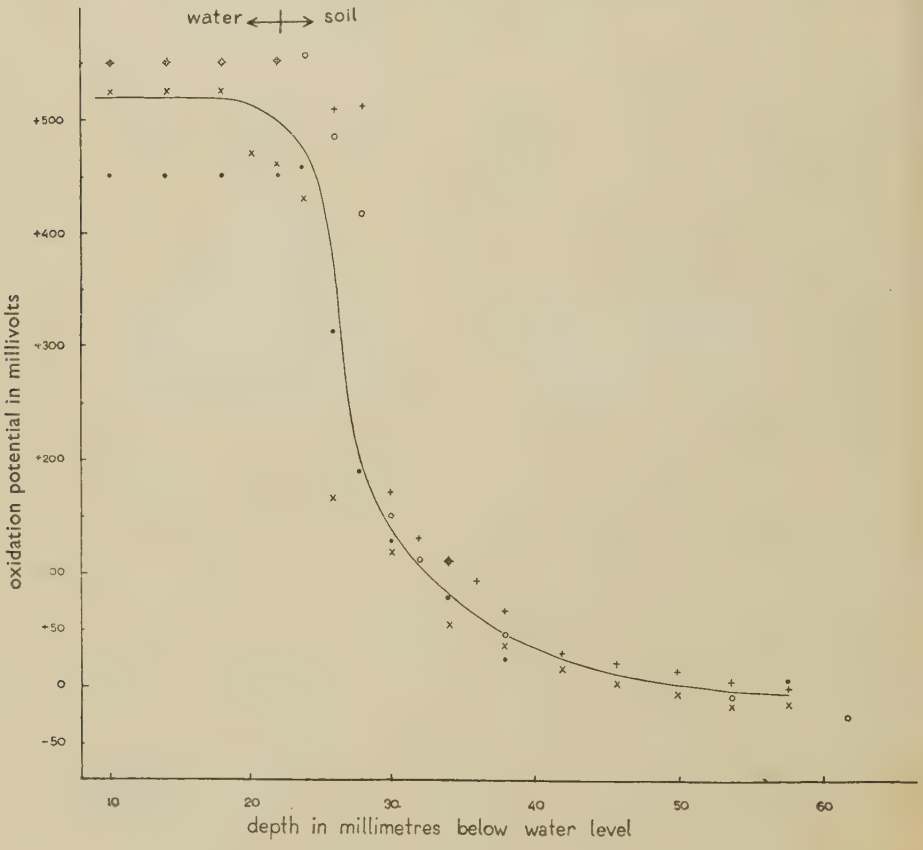

Fig. 4. Oxidation potential in irrigation water and soil at different depths below the water level (pot experiment). Four successive measurements.

the irrigation water varied, depending on the amount of sunlight and the time of day (see also table IV). But at a given time the potential was the same throughout the irrigation water. As soon as the electrode reached the soil, however, the potential immediately fell to much lower values. From the further course of the curve it

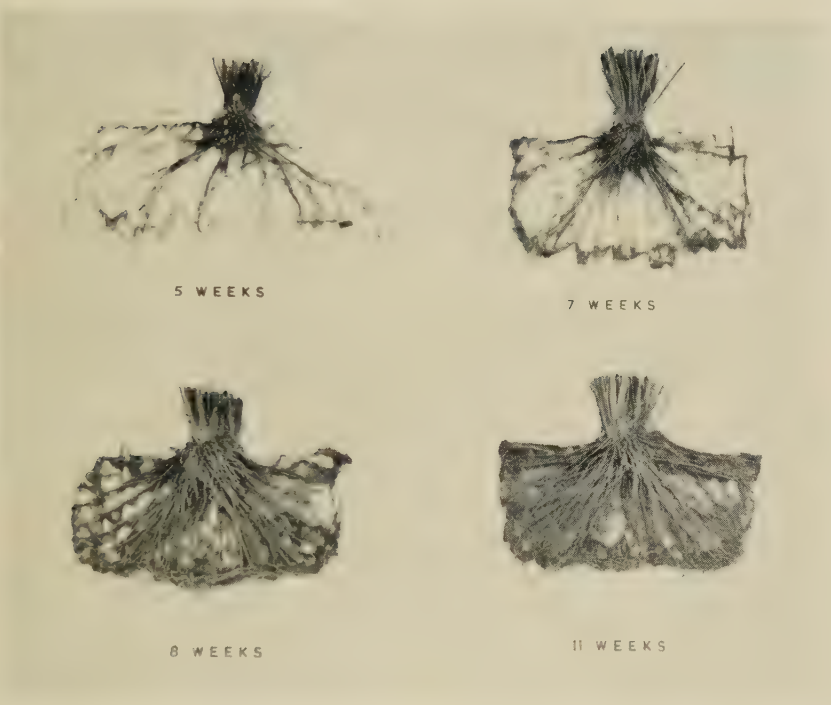

Plate I. Four stages in the development of the root system of rice.

Plate II. Adventitious roots of rice; a and c: finer type; b and d: coarser type.

appears that the values in the soil were almost the same for a given depth. As the surface of the soil was not quite flat it was not always reached by the electrode at the same depth below the surface of the water. Otherwise the agreement between the successive measurements would have been even better. Thus the results of De Gee were fully confirmed. The oxidation potentials in the pots were almost exactly the same as in the field. Only one centimetre below the surface of the soil the potential reached very low values in both cases.

Thus the vast majority of rice roots must be in a medium certainly devoid of free oxygen and if these roots are to function adequately oxygen must be supplied from other sources.

THE ROOT MAT AND ITS INFLUENCE ON THE OXYGEN SUPPLY OF THE ROOT SYSTEM

Extensive descriptions of the root system of rice have been given by van Breda de Haan[1]), Juliano and Aldama[11]), and by Kuilman[19]). However, since these authors do not mention a root mat an extension of these descriptions will not be superfluous.

As is already known, a normal adventitious root possesses branch roots, fine rootlets and root hairs. The branch roots also possess rootlets and root hairs. On the rootlets root hairs have never been observed. For a full description the reader is referred to the literature cited.

There appear to be two types of adventitious root: the normal kind, about 2 mm in diameter and a much smaller type with a diameter of about $\frac{3}{4}$ mm. Both types possess branch roots and fine rootlets but the latter type has a more extensive development of both kinds as is shown on pl. II.

The first adventitious roots, together with the main root, are of the finer type but abundant roots of the coarser type are very soon formed. The horizontal and vertical roots, which develop in the second period of growth, are of the smaller type.

However, for several reasons it is often difficult to distinguish between the two types. In the first place the formation of these types of root is influenced by external conditions (*e.g.* more roots of the finer type are present when, in the field, several young rice plants

are planted together in one hole). Secondly, the adventitious root possesses great regenerative ability. When, for instance, the adventitious root is damaged many branch roots develop just below the injury and these roots are identical with the primary root, except that they are thinner. Thus secondary roots of the coarser type can easily be mistaken for primaries of the finer type (pl. II*b*). Only by sectioning the whole root system it is possible to discriminate between the two types since differences can only be seen at the base of the roots. With plants in their second period of growth it can then be seen that roots of the thinner type usually grow horizontally while those of the thicker type grow downwards (fig. 2). When cultivating rice in a nutrient solution the thicker type of root develops almost exclusively; a root mat is never seen. This may account for the fact that other authors have not distinguished these two types of adventitious root. Only now and then roots of the thinner type have been observed, growing vertically upwards. These roots have already been observed by van Breda de Haan. He suggests that they have a function in gas exchange but he does not give a clear idea of what this function might be. They are difficult to observe since they grow for the greater part between the leaf sheath and the stem.

In order to investigate whether the root mat, described in the previous paragraphs, has a function in the oxygen supply of the whole root system the following experiments were carried out.

1. Four glazed earthenware pots were coated with paraffin wax on the inside, filled with soil and water and planted with rice in the way described earlier. At the end of the grand period of tillering, when the beginning of root mat development could be discerned, two of these pots were treated with a layer of motor oil (2 cm thick) on the surface of the water, the two other pots remaining as controls. Three times a day oxygen-free water was administered to the irrigation water of the former pots by means of a 200 cc syringe.

One month later, when the uppermost leaf — the flag — could be recognized as such and the ears were expected to be visible within a week, the oxidation potential was measured in two pots, one with oil and one without, the plants being matched for size and number of tillers. The potential was measured with a blank Pt-electrode at the end of a short glass tube since a more suitable device was not available at that time. In order to avoid contamination of the electrodes

with oil two glass tubes were placed vertically in the irrigation water and the oil removed from them. Since contact between water and air was restored during a brief period by this treatment the oxidation potential of the irrigation water may have risen somewhat. From table III it can be seen that the potentials were about equal in both pots below a soil depth of 1 cm. As the electrode was rather short the whole depth of the soil could not be measured. The potentials at the soil surface, however, differ considerably as would be expected.

TABLE III

Oxidation potentials in two earthenware pots each with one rice plant in heading stage. In one pot the irrigation water is covered with a 2 cm layer of motor oil.

depth	no oil	oil
cm	mV	mV
irr. w	+ 475	?
0	+ 475	+ 45
1	— 32	— 32
2	— 27	— 43
3	— 38	— 51
4	— 42	— 51
5	— 42	— 51
6	— 47	— 54
7	— 51	— 54
8	— 57	—
9	— 57	18

After these measurements oil and irrigation water were poured off and in the pot without oil a thin layer of very fine roots was observed at the soil surface. In the pot with oil no such roots could be seen. A repetition of the experiment gave similar results except that a root mat was present in the pots with oil. This mat was not so well developed as in the control and the roots were coloured brown.

After the soil had been washed away from a section taken as described earlier, further differences between the two root systems could be observed. Without oil, the root system resembled that described in the foregoing section, but, with oil, there were considerably less rootlets and, further, many of the adventitious roots were dark brown and water-logged. The difference between the two root systems was very marked indeed, as can be seen from pl. III.

Since the root system had already reached its maximum development at the beginning of the experiment (see p. 15) it must be con-

cluded that a significant part of it degenerated as a consequence of the oil at the surface of the water. There was always enough water between the oil layer and the soil so that the differences in root development were not caused by a contamination of the soil with motor oil.

2. To examine the possibility that the differences in the root system, described above, could have been caused by the fact that light was excluded from the irrigation water when this was covered with motor oil, one pot, planted with rice, was covered from the beginning with a wooden disk, containing a central hole for the plant.

In both the covered pot and in the uncovered control a root mat developed. In this case, however, that in the dark was much more extensive. Abundantly branched roots even crept upwards along the wall of the pot into the air and appeared between the edge of the pot and the wooden cover.

When the first ears appeared, the temperature and the oxygen content of the irrigation water were measured in both pots and the roots examined in the usual way. The differences in temperature between the two pots did not amount to more than 3°C, but the differences in oxygen content were considerable, as can be seen from tabel IV. At 2 pm the oxygen content of the irrigation water in the light was more than three times that in the dark. Later on the differences diminished gradually and at sunrise the concentrations in the two pots were equal.

TABLE IV

Oxygen content of the irrigation water of two pots:
a. no cover (light),
b. pot covered with wooden lid (dark).

date	time	oxygen content	
		light	dark
		mg/l	mg/l
5-9-'51	11.00	8.99	2.19
6-9-'51	8.00	5.02	5.03
	11.00	8.63	3.26
	14.00	9.15	2.58
	17.00	5.01	2.25
7-9-'51	8.00	4.28	2.39
	11.00	9.15	2.25

It appears from this experiment that a root mat can develop very

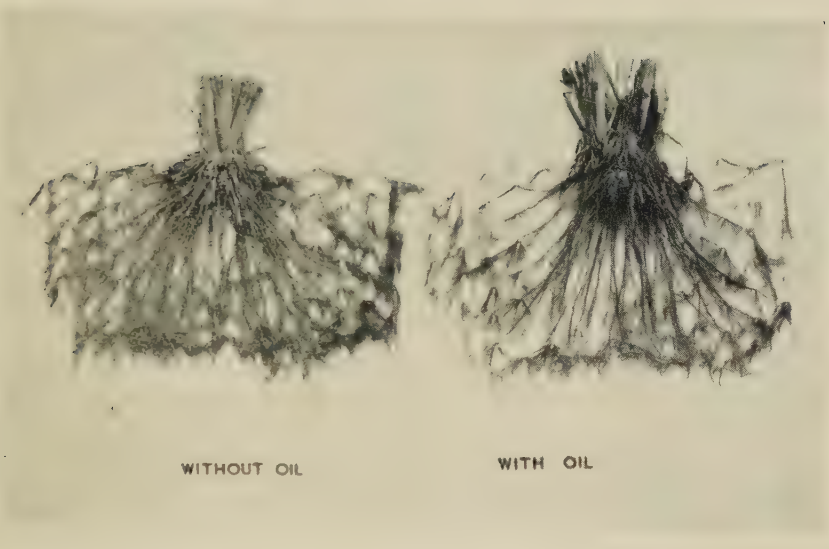

Plate III. Root system of rice.
Right: irrigation water covered with motor oil;
left: no oil on irrigation water (control).

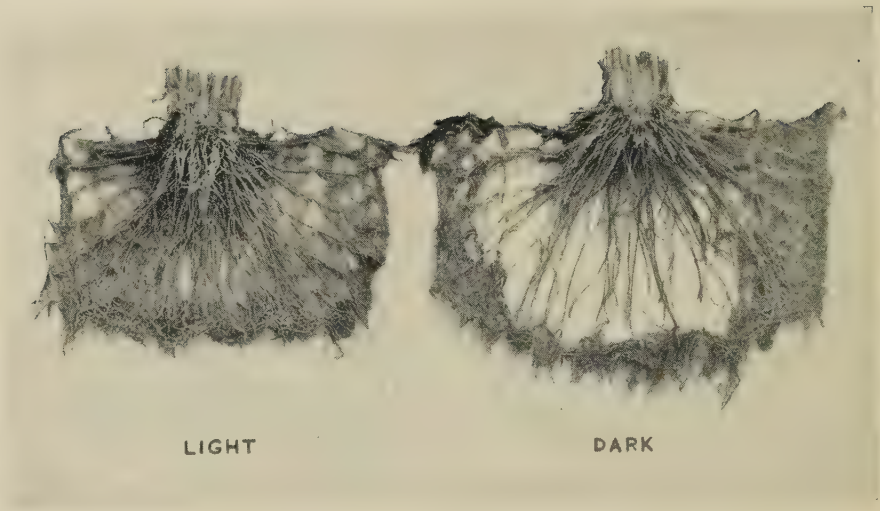

Plate IV. Root system of rice.
Right: irrigation water covered with wooden lid; left: no cover (control).

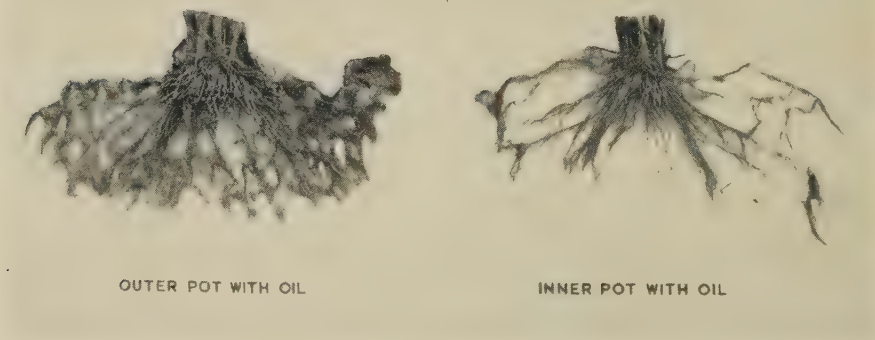

Plate V. Root development in soil of system I and system II (see text).

well in the dark and, thus, the absence of such a mat in the pots with oil is not caused by the exclusion of light.

On sectioning it appeared that the covering of the pot had been harmful to the rest of the root system (pl. IV). It may be that the oxygen concentration of the irrigation water was too low (see below).

Since the exclusion of light did not depress root mat development in the aforementioned experiment, it stands to reason that the oxidation potential must have been responsible. Below the oil layer this potential was very low and with the Winkler titration no free oxygen could be detected.

3. In order to investigate further whether the development of the root mat is dependent on the oxygen concentration the following experiment was carried out.

Two glazed earthenware pots of the size mentioned above were coated with paraffin wax on the inside, except for the bottom. The bottom contained five holes of about 2 cm diameter. Each pot was placed inside a bigger one so that when the rims were level there was a space of about 5 cm between the walls and the bottoms of the two pots. The larger pot was also glazed and coated with paraffin wax on the inside (bottom too). The bottom of the inner pot was covered with a layer of "indjuk" (fibrous tissue from the sugar palm) 3 cm in thickness and filled to a depth of about 5 cm with a mixture of sand and dried ground paddy soil in a ratio 3:1. This mixture was puddled with plenty of water. It was coarse enough to enable rapid drainage so that the water levels in the outer and inner pot were always the same. With soil alone no easy communication between the pots was possible. In the inner pots one young rice plant was planted after puddling.

As soon as the first white roots appeared in the irrigation water oil was added. In one of the two sets the oil covered the water in the inner pot (system I), and, in the other, the water in the outer (system II). In addition, the water in the outer pot of system I was aerated continuously in order to maintain an adequate oxygen concentration at the bottom. In this way system I resembled an inverted paddy field, as oxygen could penetrate the soil from below only. In both systems water was added to the pot containing no oil to compensate for losses by transpiration and evaporation.

During the further growth of the plant some determinations of the oxidation potential of the soil in both systems were made; the oxy-

gen content of the water was also measured. These results are given in table V.

TABLE V*a*

Oxygen content of the water in inner and outer pot of system I and system II (see text).

date	system I		system II	
	26-9	13-10	26-9	13-10
	mg/l	mg/l	mg/l	mg/l
inner pot	—	0.00	15.87	21.15
outer pot.	8.37	7.00	0.00	0.00

TABLE V*b*

Oxidation potentials in system I and system II (see text).

depth	oxidation potental				
	system I		system II		
	20-9	22-9	20-9	22-9	
cm	mV	mV	mV	mV	mV
0	85	65	365	365	335
1	45	65	135	165	175
2	25	45	125	105	115
3	25	45	75	85	85
4	25	35	35	75	65
5	5	25	25	65	45
6	5	25	25	55	45
7	5	25	25	45	45
8	—	—	5	45	35

Water covered with motor oil does not contain any oxygen. The aerated outer solution of system I, however, was lower in oxygen than the inner solution of system II, this difference being caused by a difference in green and blue-green algae population. The oxidation potential in the soil of both systems differed in the first few centimetres only as could be expected from other experiments (see p. 19, table III). The potential at the bottom of the pots could not be measured as no electrode of a suitable length was available. Therefore it is not known if and to what extent the potential rose at the bottom of system I.

As soon as the ears emerged the roots of both plants were examined. During the experimental period four tillers of both plants died off. This was not observed in other pot experiments and was probably caused by the fact that the soil-sand mixture was poorer in nutrients than paddy soil alone. The root systems in both pots differed considerably as can be seen from the following description.

System I: (Oil in inner pot; water in outer pot aerated).

A superficial root mat was present, but poorly developed; the roots were dark brown. The roots in the soil resembled those found in the oiled pots of experiment 1, but for the fact that in the vertical section two distinct bundles of adventitious roots could be distinguished (pl. V). There were probably five of these bundles leading to the holes in the bottom of the pot (see below). Very few branch roots and rootlets were present. At the bottom of the pot a very thick mat was found composed of all types of root. From this mat five bundles of roots protruded through the holes into the outer pot. These bundles were very thick and contained roots with many subsidiaries up to 25 cm in length. These roots, like the adventitious ones, were densely covered with long rootlets. All the roots in the outer pot were white.

System II: (Outer pot with oil).

A superficial root mat was definitely present; the roots were white and abundantly branched. The roots in the soil resembled those of the control pots in experiment 1. Roots could be found everywhere in the soil but here, too — though less distinctly — two bundles of adventitious roots could be seen as in system I (pl. V). The root mat found at the bottom of the inner pot was not nearly as thick as in system I but, nevertheless, was present. This was also the case with the roots protruding into the outer pot. These bundles were much thinner and the separate roots were more sparsely branched with shorter branch roots and rootlets. Their colour was slightly brown.

From these descriptions it can be seen that the root system in system II was quite normal, but that root development altered considerably when the direction in which oxygen penetrated the soil was reversed. In this case the roots grew through the reduced soil with little branching but branched abundantly at the boundary between soil and aerated water and in the water itself. Apparently these roots did not serve to provide the roots in the soil with oxygen as the latter were only poorly developed.

DISCUSSION

From the foregoing observations and experiments it follows that the hypothesis of S e t h i can be confirmed. The rice plant possesses special types of root which are able to take up oxygen and to trans-

port it to other parts of the root system. Thus all three forms of adaptation to a reduced medium which have been elucidated so far are present in the rice plant.

These special respiration roots, however, did not, in the present case, degenerate during the second half of the vegetation period as Sethi found. On the contrary, they started to develop at the end of the tillering stage and remained fully active until the ripening of the seed. These roots are thinner and more abundantly branched than normal roots and grow either horizontally in the irrigation water or vertically upwards into the air. Thus, both morphologically and physiologically they differ from normal roots.

In a recent publication Shah [29]) mentions a similar root mat observed in sugar cane. Varieties which possessed fine branched roots at the surface were resistant to flooding, but varieties without a root mat soon died under such conditions. Shah refers to their negative geotropy. Indeed, just as with rice, the direction of growth was against gravity but it was not proved that the gravitational field was the directive force.

Harrison and Aiyer suppose that rice roots can only develop in places where enough oxygen is available. It can be demonstrated, however, that this does not apply to the normal rice fields of Indonesia. It can be seen from the measurements of De Gees, corroborated by the present observations, that the soil is heavily reduced except for the uppermost layer of about 1 cm thickness. The bulk of the root system, however, is found deeper in the soil (pl. I). Further, if a stream of oxygenated water moves into the soil, as the authors suppose, it would make a difference in oxidation potential whether the surface of the water was covered with oil or not. In the experiments reported here no such difference was found.

The views of Harrison and Aiyer are correct in so far as the roots in a medium rich in oxygen are more abundantly branched than in a reduced medium. However, under normal circumstances these roots form a very small part of the whole root system. In the last experiment of the previous section, where the normal situation was reversed, the roots developed almost exclusively in the oxygen-rich water below the soil. In the soil itself the roots which had not reached the water degenerated when the surface was covered with oil. Apparently the roots protruding into the water were unable to keep the rest of the root system in a healthy condition as is the case

under normal circumstances. This can be explained on the grounds that the oxygen had to diffuse along a great distance to reach the stem base from which it could enter the roots growing exclusively in the soil.

Although it has been demonstrated that the greater part of the root system degenerated when the irrigation water is covered with oil, no harmful influence on the upper parts was observed. Compared with the control plants no difference in general form, height, and number of tillers could be found. Apparently the covering does not mean an immediate and complete cessation of the oxygen supply to the roots. In that case active salt uptake would become impossible and this would certainly influence shoot development. Thus, oxygen transport through the plant must be possible, to some extent, even after stem elongation. Since many roots degenerate, the system as a whole will, perhaps, be hampered in its function but it must be realized that during the first half of the growing period normal conditions prevailed. Further, the root system was only examined at the end of the vegetative period, and it is possible that degeneration started a considerable time after the application of oil.

The same conclusion as to the oxygen supply can be drawn from the experiments in culture solution. Here, again, stem elongation does not, of necessity, mean a complete blocking of the oxygen transport through the plant. In many cases the oxygen concentration in the roots of old plants was as high as it was with young plants. However, under certain conditions a heavy reduction of the solution occurred and the oxygen concentration in the roots was then very low.

Van Raalte has demonstrated that the composition of the root gas does not alter when plants are placed in an artificially reduced medium. Therefore, when a reduction occurred in the present experiments, gas transport through the plant must have been impeded. This can hardly have been caused by something other than stem elongation. Certainly this was the most conspicuous phenomenon after the tillering stage.

It could be argued that if this is so the oxygen content of the root gas after stem elongation must always be low. There are, however, other possible ways of transport.

In the first place aerial roots have been observed on plants grown on culture solution.

Secondly, in many cases roots were formed at the nodes and were, for the greater part, concealed between the stem and the leaf sheath.

In the third place it was often observed that after stem elongation many partly developed tillers were present which were concealed in the same manner.

These tillers, and both kinds of root mentioned, provide shorter paths for oxygen to enter the roots. It must be borne in mind, however, that it has not yet been established that plants which cause a reduction of the culture solution possess less air roots, node roots, and young tillers than plants which do not. This point requires further investigation.

We may, then, draw the general conclusion that, by the time of stem elongation, oxygen transport through the plant itself is hampered, to an extent depending on developmental factors which are not yet fully understood. Development of a root mat at the soil surface facilitates oxygen transport to the rest of the root system but does not necessarily imply that other ways of oxygen supply are completely blocked.

SUMMARY

With rice cultivated in a nutrient solution it was observed that after stem elongation severe reduction often occurs in this solution. The oxygen content of the gas in the root cortex of such plants appeared to be very low. The reduction decreased when a young rice plant was introduced into the same vessel together with the old one.

It was found that rice plants in the field develop a superficial mat of fine, abundantly branched, roots at the surface of the soil.

It could be shown by pot experiments that this root mat starts to develop at the end of the tillering period. The roots are thinner and more abundantly branched than those growing deeper in the soil.

By covering the irrigation water with a layer of motor oil only a poorly developed root mat was formed, while the greater part of the rest of the root system degenerated. However, no harmful effects on the upper parts were found. When a pot was covered with a wooden lid a very extensive root mat developed; the poor development with oil, therefore, cannot have been caused by the mere exclusion of the light.

In normal pots the oxidation potential diminished sharply below a soil depth of about 1 cm. When the surface of the irrigation water was covered with oil the potential was low throughout the soil and the irrigation water. In addition, under such circumstances no oxygen was found in the irrigation water.

It is concluded from these experiments that the rice plant possesses

special respiration roots which come into play after stem elongation. Oxygen transport through the plant itself is then somewhat restricted so that, in a nutrient solution, reduction may occur. The extent to which transport is retarded depends on developmental factors which are not yet fully understood.

Received February 27, 1953.

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Plant and Soil V, no 1 October 1953

THE INFLUENCE OF NITROGEN ON THE GROWTH OF JAPANESE LARCH (*LARIX LEPTOLEPIS*)

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INTRODUCTION

The growth of Japanese larch, in contrast with other tree species used in Dutch forestry, is not stimulated by nitrogen. Results of site and yield investigations at the Forest Research Station T.N.O. even point to a definite decrease in growth with increase in nitrogen [1]). Liming, equivalent in forestry to fertilizing with nitrogen since it stimulates the decomposition of the raw humus, also results in lower yields [5]) [6]). Fertilizing with phosphates, however, seems to improve growth.

This reaction of Japanese larch to nitrogen is probably related to its ecological character. The natural range of the genus Larix coïncides with the podzol zone of the northern hemisphere and several mountainous regions [2]). Larch is a pioneer tree species in some forest associations of this range. A u e r [3]) in phytosociological investigations determined that larch in Switzerland is optimally developed at the pioneer stage of the succession to the Rhodoreto-Vaccinietum-Cembretosum. In older stages of the succession, where the soil is incorporated with more humus larch does not regenerate and is replaced by *Pinus cembra*. In the Alps the natural occurrence of larch is associated with calamities such as landslides, fire, and so on. These calamities cause a regression of site development to the pioneer stage. The optimum occurrence of larch on non podzolised soils, is certainly to be considered as an indication of the low requirement for nitrogen of this tree species.

The abnormally high C/N ratio of the litter is more or less in accordance with this. However, the investigations of S a t o [8]) into

the physiology of the nutrition of Japanese larch point to a stimulating effect of nitrogen.

The influence of nitrogen and phosphate on the growth of *Larix leptolepis* has been determined by means of pot culture tests. The results of these tests are described below and are considered in connection with the phenomenon mentioned above.

EXPERIMENTAL

The pot cultures have been established with three kinds of soil, the mechanical composition of which was more or less similar (loamy sand). Their contents of nitrogen and phosphate, however, differ greatly; the content of humus in soil 351 amounts to 0,5%, the other soils contain 5,5%. Other data are given in Table I. Soil 351 is poor in nitrogen and phosphate, soil 376 is rich in nitrogen and poor in phosphate, soil 359 is rich in both nitrogen and phosphate.

TABLE I

Composition of soils used			
soil	pH H_2O	N tot. in %	P tot. in %
351	5,3	0,010	0,018
376	4,2	0,115	0,037
359	4,2	0,112	0,100

Each pot — an ordinary flower pot, covered inside with paratol — was filled with 4000 grams of soil and planted in May with 13 seedlings of Japanese larch. From May to September 1500 cc of nutrient solution were supplied. The composition of this nutrient solution was determined according to Björkman[4]). Each series contained 16 treatments, 4 levels of nitrogen in the form of NH_4NO_3 and 4 levels of phosphate, as H_3PO_4, singly and in combination. The quantities of NH_4NO_3 and H_3PO_4 in mg per liter are given in Table II.

TABLE II

Composition of the nutrient solution			
	mg NH_4NO_3/l		mg H_3PO_4/l
O	0	O	0
N_1	285,6	P_1	457,0
N_2	571,2	P_2	914,0
N_3	1142,4	P_3	1828,0

In September the production of organic matter, the amount of total nitrogen and total phosphate in the green needles were determined.

RESULTS

a. *Growth of the larches in relation to nitrogen and phosphate.*

The average dry weight of shoots in mg per plant per pot is used as a standard for growth. These results are given in Table III.

TABLE III

Dry weight of shoots per plant												
Soil	351				376				359			
nutrient supply	O	N_1	N_2	N_3	O	N_1	N_2	N_3	O	N_1	N_2	N_3
O	30	40	30	25	60	63	46	40	74	77	85	98
P_1	30	69	85	69	132	140	102	96	99	129	150	155
P_2	24	87	92	74	153	157	113	88	118	131	138	144
P_3	16	77	90	74	137	136	85	65	114	118	130	140

It is clear that the soil has an important influence on the growth of the larches and their reaction to nitrogen. In soil 351, poor in nitrogen and phosphate, growth is increased by small quantities of nitrogen when given in combination with phosphate. A greater supply of nitrogen decreases growth. Phosphate always stimulates growth, except in the absence of added nitrogen. There exists an obvious positive interaction between nitrogen and phosphate. In soil 376 — poor in phosphate and relatively rich in nitrogen — this interaction is negative. Phosphate stimulates growth considerably, while on the other hand nitrogen decreases growth. When the soil is rich in both components, nitrogen and phosphate — soil 359 — the interaction is also negative; in this case, however, nitrogen does not decrease growth, but even increases it to a small extent.

b. *Absorption of nitrogen and phosphate by larch.*

Leaf analyses most clearly demonstrate the absorption ratio of the nutrients. In Table IV the results of the analyses of the needles are given.

TABLE IV

Nitrogen and phosphate content of the needles as % of dry weight												
Growth factor	Soil	351				376				359			
	Supply of nutrients	O	N_1	N_2	N_3	O	N_1	N_2	N_3	O	N_1	N_2	N_3
N	O	0,96	4,50	5,66	6,17	2,87	3,92	4,12	4,88	2,75	3,49	4,01	4,13
P		0,70	0,43	0,42	0,39	0,43	0,36	0,14	0,14	0,59	0,43	0,43	0,43
N	P_1	1,01	2,48	2,91	3,56	2,65	3,19	3,41	3,75	2,55	3,11	3,27	3,59
P		1,80	0,64	0,63	0,60	0,96	0,68	0,69	0,68	1,34	1,18	1,08	0,95
N	P_2	1,26	2,50	2,77	3,45	2,16	2,88	3,15	3,47	2,50	2,86	3,35	3,43
P		2,12	0,76	0,63	0,65	0,95	0,62	0,58	0,43	1,28	0,94	0,94	1,00
N	P_3	1,04	2,25	2,80	3,38	2,41	2,95	2,95	3,10	2,44	2,70	3,19	3,36
P		3,23	1,07	0,73	0,83	0,88	0,62	0,42	0.40	1,27	0,94	1,08	0,87

These data suggest the existence of an antagonism between nitrogen and phosphate, which is not attributable to differences in growth [7]).

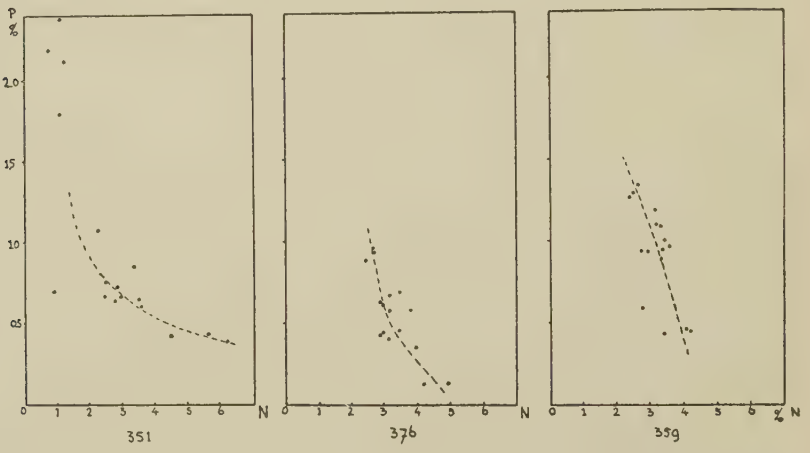

Fig. 1. Phosphate content of needles plotted against nitrogen content. From left to right: soil 351, poor in N and P; soil 376, rich in N and poor in P and soil 359, rich in N and P.

In all soils the absorption of phosphate is decreased by an increase in nitrogen absorption and vice versa. The nitrogen and phosphate contents of the needles are plotted in Figure 1. This figure obviously demonstrates the N/P-antagonism. In soil 351 the contents of nitrogen and phosphate are most divergent: the variations of nitrogen content are considerably lower in soil 376 and lowest in soil 359. Even in this last soil, which is rich in phosphate, the absorption of this growth factor is decreased by nitrogen. For a clear demonstration, the data for growth and leaf analyses are plotted against the N- and P supplies in three dimensions in Figure 2.

c. *N/P ratio and growth.*

There appears to be a close correlation between N/P ratio of needles and growth of larch. The N/P ratios are given in Table V.

In Figure 3 these N/P ratios are plotted against the growth values for the three series. Characteristic for all series is an abrupt optimum of growth over a narrow N/P range from about 4 to 5. On both sides of this sharp optimum growth decreases rapidly. In soil 351 there is

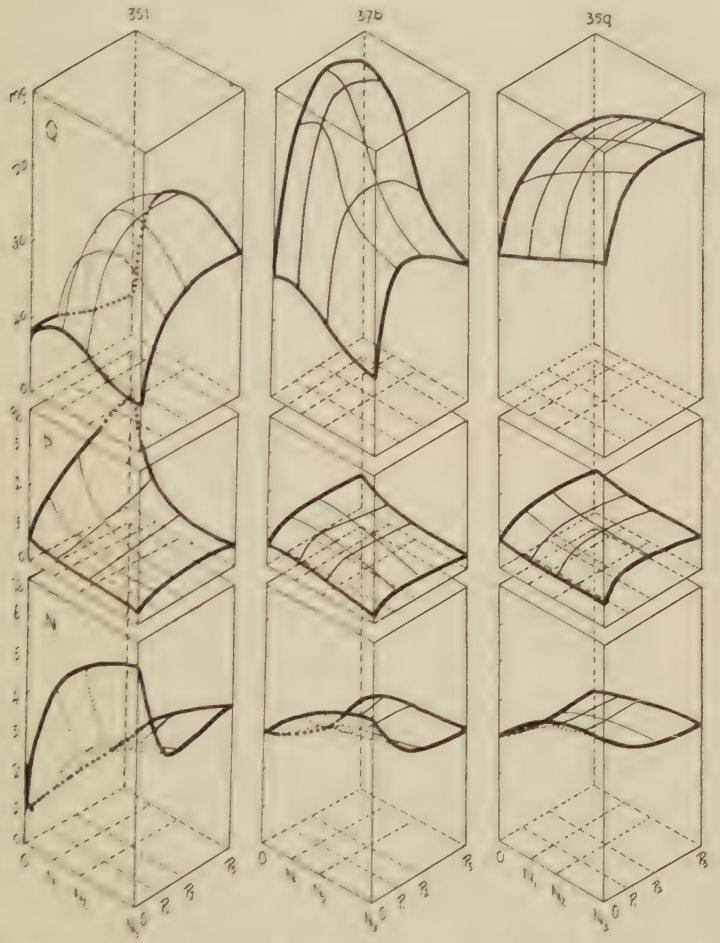

Fig. 2 Average dry weight of shoots, P content and N content of needles, three-dimensionally plotted against N and P supplies, for soils 351, 376 and 359 respectively.

TABLE V

N/P ratio of the needles												
soil	351				376				359			
supply of nutrients	O	N_1	N_2	N_3	O	N_1	N_2	N_3	O	N_1	N_2	N_3
O	1,4	10,4	13,4	15,8	6,6	10,9	29,4	34,9	4,7	8,4	9,8	9,6
P_1	0,6	3,9	4,6	5,9	2,7	4,7	4,9	5,5	1,9	2,6	3,0	3,8
P_2	0,6	3,3	4,4	5,3	2,2	4,6	5,4	8,0	1,9	3,0	3,6	3,4
P_3	0,3	2,1	3,8	3,9	2,7	4,7	7,0	7,7	1,9	2,9	3,0	3,8

a great variation in the N/P ratio, due to the dominating influence of the nutrients supplied. In soil 376 the effect of the nitrogen supply is increased by the nitrogen content of the soil, the N/P ratios are displaced to the right of the optimum value and growth decreases. In soil 359, however, much phosphate is available, so that the N/P ratios are mainly displaced directly around or to the left of the optimum value.

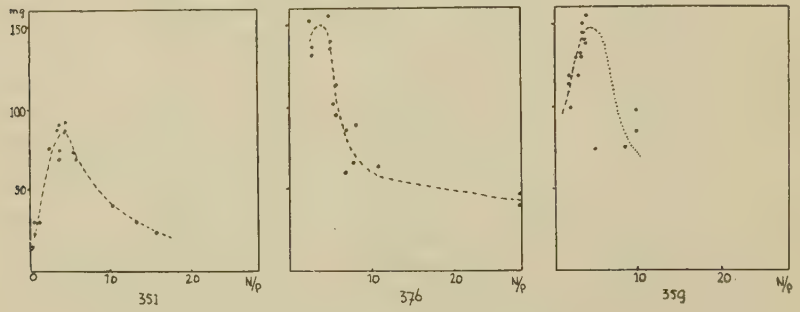

Fig. 3. Average dry weight of shoots plotted against N/P ratio of needles, for soils 351, 376 and 359 respectively.

CONCLUSIONS

The results of the pot culture experiment point to the existence of a N/P antagonism in Japanese larch. Nitrogen decreases the intake of phosphorus, and phosphate decreases the absorption of nitrogen. The growth reaction, however, is dependent on the composition of the soil with regard to its content of nitrogen and phosphate. Growth is optimal over a narrow N/P ratio range and decreases rapidly on both sides of this optimum. Therefore the poorer the soil is in phosphate, the more rapidly will growth decrease as the supply of nitrogen is increased. In a soil rich in phosphate, absorption of this nutrient will be decreased by nitrogen, but, because of the availability of excess phosphate, the N/P ratio in the needles will not increase much above the optimum. In such cases growth can be increased by nitrogen. In contrast to nitrogen, phosphorus does not have a harmful effect on the growth of larch, except only when little nitrogen is available. This is an indication of the low need for nitrogen, which is in accordance with the ecological character of the larch. Optimum development as a pioneer tree species on undeveloped podzols is only possible with this property. The decrease in growth

with increasing nitrogen content of forest soils, which are normally poor in phosphate, is also in agreement with this. The harmful effect of liming — corresponding to increase in the amount of available nitrogen — and the neutralization of growth disturbances by phosphate fertilizing can be considered a result of this low need for nitrogen and the N/P antagonism in Japanese larch. These qualities involve important consequences concerning the judgement of the suitability of a site for Japanese larch and the necessity of fertilization. The criteria to be applied deviate fundamentally from those normally used in forestry.

Received April 2, 1953.

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Plant and Soil V, no 1 October 1953

RADIATION EFFECTS WITH RADIOACTIVE PHOSPHORUS IN BARLEY AND TOMATO GROWN IN SOIL

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INTRODUCTION

Before radioactive isotopes can be used in plant nutrition experiments, it is important to establish whether radiation from the disintegration of the tracer affects the metabolism of the plants. Radiation effects, should they occur, may or may not be sufficiently great to invalidate the main treatment differences being investigated.

Radioactive phosphorus (P^{32}) has been extensively used as a tracer both in water and soil cultures and in many investigations consideration has been given to radiation injury.

Arnon [1]) did not observe injurious effects in young barley seedlings grown in nutrient solution which contained 92 μc P^{32} per litre initially. Spinks *et al.* [14]) germinated wheat, barley and sunflower seeds in aqueous solution, and later transferred the seedlings to a P^{32} solution. Seeds which had absorbed 1.4 μc P^{32}/seed failed to develop. Russell and Martin [11]) grew young barley plants in aqueous solution low in phosphate and later transferred them to a radioactive phosphate solution. They observed a significant reduction in root weight, and at 50 μc P^{32}/litre, the total P content of the roots was reduced within six days to 40 per cent of those grown in solution containing 5 μc P^{32}/litre. Blume *et al.* [2]) grew barley in nutrient solutions similar to those used by Russell and Martin [11]). They found small changes only in dry weights, and no effect on the phosphate absorption by the plants, despite the use of considerably higher specific activities of tracer than those used by Russell and Martin.

Conflicting results have also been reported in soil. Hendricks and Dean [6]) failed to find any difference in the above-ground parts of rye grass treated with 75, 375 and 1875 μc P^{32} per pot respectively. Dion *et al.* [5]) in field experiments with wheat claimed that no harmful effect due to tracer had occurred. Pot culture experiments with soil have also been carried out by Russell, Adams and Martin [12]) who suggested that a change in the proportion of fertiliser to soil phosphorus absorbed provided an indication of tracer injury. For spring sown barley harvested 39 days after sowing, this ratio for the 4 μc P^{32} per pot treatment was significantly lower than at the lowest tracer level used (0.16 μc per pot). For winter wheat however harvested 70 days after sowing, the ratio at 5 μc P^{32} per pot was significantly higher than at 2 μc P^{32} per pot. These data are further discussed by Russell *et al.* [13]). Blume [3]) using specific activities ranging up to 12,500 μc P^{32}/g P^{31} observed effects on dry weights, total phosphorus etc., which though statistically significant were considered to be too small to affect the main experimental result.

Thus there appears to be considerable differences in the results obtained by various workers in regard to radiation injury in plants. Before using P^{32} in plant nutrition studies, it was necessary therefore to determine whether radiation effects occurred under our experimental conditions. This paper amplifies a preliminary publication by Bould, Nicholas and Thomas [4]) in which the magnitude of radiation effects was determined in tomato and barley grown in two types of soil in well replicated pot experiments. Two levels of fertiliser phosphate, supplied as sodium dihydrogen orthophosphate equivalent to about 200 and 500 lb. superphosphate per acre of soil, were labelled with a range of P^{32} activities from 0.5 to 500 μc per pot. The dry weights, total phosphate content, fertiliser and soil phosphate uptakes were determined in tops and roots respectively and also various ratios derived from these data were calculated. We define radiation effects as changes in any of these quantities that can be attributed to the tracer. No attempt has been made to study the cytological changes that may have occurred in our experiments, as they do not directly concern our investigations.

EXPERIMENTAL

Glasshouse Procedure

Preparation of Pot Experiments. The 6″ earthenware pots and saucers used in the factorial experiments were washed, and treated with bitumen. The soil was air dried, sieved through a $\frac{1}{4}$-inch mesh steel sieve, and a known weight (about 2 kg) transferred into each pot. The two plants chosen were barley, variety Spratt Archer, and tomato, variety Market King, since both are good indicators of mineral deficiencies (Wallace[17]). In Experiments 1, 2 and 4, ten barley seeds were sown at a depth of $\frac{1}{2}$″ in each pot. In Experiment 3, one tomato seedling, three weeks from seed, was transplanted into each pot containing the test soil. Prior to transplanting the potting soil was removed from the seedling roots to ensure that subsequent phosphorus uptake would be from the test soil only. The total P contents of barley seed and that of spare tomato seedlings were determined at this stage. These values were used as a correction in calculating soil P uptake.

Preparation and Application of Nutrient Solutions. A supplementary nutrient solution was added a few hours of sowing the barley seed and within a few days of transplanting the tomato seedlings. The solution contained per litre, 5.6 g K_2SO_4; 5.6 g $MgSO_4.7H_2O$, and 11.2 g $(NH_4)_2SO_4$. 50 ml of this solution, given to each pot, was poured evenly over the surface soil.

The solution of radioactive sodium dihydrogen phosphate was supplied by A.E.R.E. Harwell. Up to 32 millicuries of P^{32} was obtained for each experiment. In all cases the chemical form of the P^{32} as orthophosphate was confirmed before use (Thomas and Nicholas[15]).

Each experiment involved the preparation of up to a dozen stock solutions at two carrier levels (equivalent to 200 and 500 lb. superphosphate per acre) and each carrier level was labelled with up to six different levels of P^{32}. For convenient dispensing the stock radioactive solutions were made up in aspirators.

The six P^{32} levels at the two carrier levels used in Experiment 1 are shown below.

Stock solutions	Phosphate content per 50 ml per pot	
A	0.210 g $NaH_2PO_4.2H_2O$	+ 400 μc P^{32} as $P^{32}O_4$
C	,,	150 μc ,,
E	,,	25 μc ,,
G	,,	5 μc ,,
J	,,	1 μc ,,
L	,,	nil
B	0.084 g $NaH_2PO_4.2H_2O$	+ 400 μc P^{32} as $P^{32}O_4$
D	,,	150 μc ,,
F	,,	25 μc ,,
H	,,	5 μc ,,
K	,,	1 μc ,,
M	,,	nil

50 ml of the appropriate stock solution was poured evenly from a graduated cylinder over the soil. The phosphate solution was applied to barley seed a day after sowing and to the tomato a week after transplanting into the test soil. To minimise contamination, the pots for a given phosphate treatment were brought to the dispensing bench and treated. They were then replaced and the next group dealt with.

Sampling Methods. The plants were harvested 3, 6 or 9 weeks respectively after the addition of the phosphate solution. They were sampled in ascending order of P^{32} activity starting with the control treatment. The bulk of the soil was removed from the roots by transferring the plant plus the soil from the pot to a basket made of fine wire mesh. The basket was gently raised and lowered in a bucket of water containing a detergent (ester salts, 1 fluid oz. per 10 galls. of water). After most of the soil had been removed (2 min) the plants from one pot, viz. 10 barley or 1 tomato plant, were placed on a perforated zinc tray (18″ long and 6″ wide) in the sink, and the remainder of the soil removed by washing with a fine jet of water, a procedure which minimised mechanical injury to the roots. The washing process took less than 5 min per sample. When 2 or 3 pots were combined to form a unit treatment, the same number of sinks were used, each manned by an operator. In this way variations in washing procedure between operators was accounted for.

After thorough washing, the plants were cut at soil level into 'tops' and 'roots', put into weighed beakers and dried at 96°C to constant weight.

It was suggested, by Dr R. S. Russell (private communication) that root washing could result in loss of phosphate by leaching and that this could be considerably reduced by pre-treating the soil with lead acetate solution. The results of extensive tests showed that the P content of the plants treated with lead acetate was not statistically different (1% level) from that of the non-treated plants when given a similar washing treatment. The lead acetate treatment of the soil was therefore not adopted.

Laboratory Procedure

Plant digestion. The dried plant material after weighing was acid digested using 15 ml HNO_3 (A.R.) per g of dry matter. After cooling 2 ml $HClO_4$ (A.R.) per g of dry matter were added and digestion continued until oxidation was complete. After hydrolysis of lower phosphates to orthophosphate by adding distilled water and boiling for 15 min the solutions were cooled and made up to volume. The radioactive solutions were radio assayed and then the digest solutions were analysed colorimetrically for total orthophosphate (Kitson and Mellon[7]).

Radioactive Assay. Radioactive assay of the plant digests was made by solution counting in a M.6 tube designed by Veall[16]), shielded from extraneous radiation and light in a suitably designed 'lead castle' having lead walls 1½″ thick.

The digest solutions from each P^{32} group were counted in turn using the

appropriate radioactive stock solutions as the standard. The standard stock solution was counted after every two or three digests, and in this way radioactive decay of P^{32} and also small variations in counter efficiency were allowed for.

The scaler used was a model 22 GM counter with a scale of 32 supplied by Panax Equipment Ltd., modified to incorporate a quench circuit. The preferred range of counting rates was between 1500 and 2500 counts per min, for which the dead time correction never exceeded 1% of the counting rate. Where practicable counting was carried out to give a statistical precision of under 1%, but with very weak solutions, counts with a statistical error of about 2% were accepted.

Maintenance of M.6 Tubes. After each count the M.6 tubes were cleaned and dried in a routine manner as recommended by Veall[16]). The efficiency of the cleaning method was confirmed by background determinations which usually varied between 10 and 14 counts per min. The M.6 tubes are occasionally photosensitive so that all counts were made in the dark by keeping the top of the 'lead castle' closed. The laboratory temperature was maintained between 60° and 65°F.

Computation of Results. The following calculation illustrates the method used for evaluating the fertiliser phosphate uptake by a plant which had been treated at the beginning of the experiment with 50 ml of radioactive stock solution C, which also contained W g P^{31} as orthophosphate.

The absolute efficiency of the M.6 tube used for the counting need not be known, but for this calculation it is assumed to be such that a solution containing 1 microcurie of P^{32} per litre gives x counts per minute. (The actual value of x is approximately 1900 counts per minute).

Solution counted	Counts per min determined on the same day (both corrected for background)	P^{32} concentration
Plant digest (tops) in 100 ml	b	b/x μc P^{32}/litre or $b/10x$ μc P^{32}/100 ml
Radioactive stock solution C, (50 ml of which had been supplied to the plant at the commencement of the experiment)	a	a/x μc P^{32}/litre or $a/20x$ μc P^{32}/50 ml

∴ % of fertiliser phosphate supplied to the plant at commencement of the experiment which is present in the plant 'top' at harvest

$$= \frac{b/10}{a/20} \cdot 100 = 200b/a$$

∴ Weight of fertiliser P^{31} supplied which is in the plant 'top' at harvest

$$= 2bW/a \text{ g}$$

Safety Precautions

Since the quantities of radioactive material handled in these experiments were necessarily high (up to 32 millicuries), a safety procedure was adopted to ensure that the radiation dosage received by the workers was below the tolerance recommended by the Medical Research Council. The most hazardous operation involved the preparation of the stock solutions from the concentrate received from Harwell. This was done behind a glass screen in a preparatory laboratory adjacent to the Glasshouse. Another potentially hazardous operation involved the bulking of dried and dusty plant material. This was carried out in a fume chamber behind glass screens. Rubber gloves and eye glasses were always used as a routine protective measure.

All the pot culture work was carried out in a large glasshouse specially designed for radioactive work. There were three benches in the house, each bench about one foot above the floor to facilitate watering. Two tables at one end of the house were used for dispensing the stock solution to the pots. The floor of the house was made of cinders, so that spillage of radioactive solution from an aspirator could be easily dealt with by removing the contaminated parts. A large wash basin with a foot-operating tap was also included. Power points were available at both ends of the house so that the monitor (Panax Model 40A) could be used wherever required. A monitor check was made on all operations from which the hazard could not be completely eliminated.

To confirm the efficiency of the precautions adopted, all those involved in the radioactive work had blood counts at 3 monthly intervals. This was arranged with the co-operation of Professor Perry at the Bristol Royal Infirmary. In addition, film badges supplied by the National Physics Laboratory were worn by each person. After a week's use these were returned to the N.P.L. for assessment. In no case was the dose above the minimum which the N.P.L. record quantitatively. It was concluded therefore that the handling precautions were adequate.

EXPERIMENTS

The details of experiments 1 to 4 are given in Table I.

RESULTS

The following data are given for barley and tomato respectively in each of the four experiments; dry weights of the plants above and below ground respectively, their total phosphorus content, fertiliser phosphate uptake, fertiliser phosphate uptake/soil phosphate uptake, fertiliser phosphate uptake/total phosphate, fertiliser phosphate in tops/fertiliser phosphate in roots. In the statistical analysis of the data, effects significant at the 1% level only are considered. Values significantly different from the controls or from the lowest radioactive levels of P^{32} are indicated by heavy type in Tables II–VI.

TABLE I

Experimental details				
Experiment No.	1	2	3	4
Crop	Barley var. Spratt Archer	As for Experiment I	Tomato var. Market King	As for Experiment I
Soil	Medium loam derived from New Red Sandstone, pH 6, citric soluble P_2O_5, 0.03%	As for Experiment I	As for Experiment I	Sandy loam derived from the Ross series, pH 5.0.
Layout	144, 6″ pots randomised in 4 blocks	120, 6″ pots randomised in 4 blocks	As for Experiment 2	72, 6″ pots in 6 blocks
No. of pots per sampling unit	3	3	1	2
P^{31} application	NaH_2PO_4 at two levels equivalent to 200 and 500 lb. Superphosphate per acre.	As for Experiment I	As for Experiment I	NaH_2PO_4 at 500 lb. Superphosphate/acre.
P^{32} levels as μc per pot	0, 1, 5, 25, 150 and 400 respectively.	0, 0.5, 4, 25 and 500 respectively.	As for Experiment 2	0, 1, 5, 25, 125 and 400
Barley seeds sown or tomato transplanted into test soil	Feb. 28th	Oct. 19th	June 27th	Sept. 4th
Application of tracer solution	March 1st	Oct. 20th	July 2nd	Sept. 5th
Sampling	March 22nd	Nov. 14th	August 12th and Sept. 2nd	Sept. 28th

Experiment 1

Statistical analysis of the data given in Table II, indicate that at certain levels of P^{32} treatment, barley plants show significant increases in dry weights of 'tops', and 'roots' and 'tops' combined. The fertiliser P uptake/total P, and fertiliser P uptake/soil P uptake are also affected by 'tracer' at 400 μc P^{32} only. The other functions are not affected.

Significant differences in dry weights are shown even from low radioactive levels viz. 5 μc per pot. The effects do not show a regular increase with increasing tracer level. It is noteworthy that the dry weights of the roots are not significantly affected by the 'tracer'.

[b]*Experiment 1*

TABLE II

Effect of P^{32} levels on dry weights, total P, fertiliser P and soil P, and ratios of these in barley (mean of 8 plots, 1 plot = 3 pots)

Functions	P^{32} as μc per pot						S.D.
	400	150	25	5	1	0	at P=0.01
Dry weights (g)							
Roots	1.23	1.24	1.19	1.29	1.28	1.22	NS
Tops	2.76	**2.91**	**2.85**	**2.89**	2.60	2.56	0.206
Roots & Tops	4.00	**4.14**	4.04	**4.18**	3.87	3.79	0.296
Total P (mg)							
Roots	6.35	6.15	5.94	6.68	5.96	6.15	NS
Tops	14.26	14.42	13.76	15.26	13.94	14.16	NS
Roots & Tops	20.61	20.57	19.70	21.94	19.90	20.30	NS
Fertiliser P (mg)							
Roots	2.03	2.08	2.04	2.19	1.92	—	NS
Tops	4.96	5.32	5.17	5.47	5.00	—	NS
Roots & Tops	6.99	7.40	7.21	7.67	6.92	—	NS
Soil P (mg)							
(Roots & Tops) O	8.80	7.87	7.46	8.91	7.50	—	NS
+	7.78	7.85	7.19	9.00	7.64	—	NS
$\log_e \frac{\text{Fert. P (tops)}}{\text{Fert. P (roots)}}$	0.87	0.92	0.91	0.90	0.93	—	NS
$\log_e \frac{\text{Fert. P uptake}}{\text{Total P}} \times 10$ (Roots & Tops)	**1.16**	1.22	1.24	1.20	1.21	—	0.041
$\log_e \frac{\text{Fert. P uptake}}{\text{Soil P uptake}} \times 10$ (Roots & Tops)	**2.06**	2.18	2.23	2.10	2.17	—	0.095
% Fert. P uptake	8.4	9.0	8.80	9.4	8.6	—	NS

O — high level of phosphorus 31

\+ — low ,, ,, ,, ,, Means of 4 plots.

The interaction between P^{31} and P^{32} was not significant except for soil P (roots & tops combined). Values significantly different from the controls in heavy type (bold).

The fertiliser phosphate and total phosphate content of the roots and tops respectively and in the total plant show no significant differences with increasing 'tracer' level. The ratio of fertiliser phosphate in tops/fertiliser phosphate in the root and percentage fertiliser phosphate absorbed was not affected.

Experiment 2

The results of the experiment with barley are given in Table III. No effects were observed in the dry weights of tops and roots singly or in combination.

Experiment 2

TABLE III

Effect of P^{32} levels on dry weights, total P, fertiliser P and soil P, and ratios of these in barley (mean of 8 plots, 1 plot = 3 pots)

Functions	P^{32} as μc per pot					S.D.
	500	25	4	0.5	0	P = 0.01
Dry weights (g)						
Roots	0.52	0.52	0.44	0.47	0.48	NS
Tops	0.92	1.00	0.88	0.87	0.93	NS
Roots & Tops	1.44	1.52	1.32	1.34	1.41	NS
Total P (mg)						
Roots	**2.16**	2.12	1.79	1.90	1.84	0.310
Tops	4.92	4.64	**4.06**	4.88	5.27	0.778
Roots & Tops	7.09	6.76	5.86	6.78	7.11	NS
Fertiliser P (mg)						
Roots	0.38	0.49	0.41	0.40	—	NS
Tops	1.07	1.46	1.19	1.24	—	NS
Roots & Tops	1.45	1.95	1.60	1.64	—	NS
Soil P (mg)						
(Roots & Tops) . . .	2.46	1.60	**1.37**	2.05	—	0.486
$\log_e \frac{\text{Fert. P uptake}}{\text{Total P}} \times 10$ (Roots & Tops) . .	2.61	2.87	2.67	2.68	—	NS
$\log_e \frac{\text{Fert. P uptake}}{\text{Soil P uptake}} \times 10$ (Roots & Tops) . .	1.73	2.41	2.38	2.00	—	NS
% Fert. P uptake . .	1.76	2.29	2.00	1.88	—	NS

Interaction between P^{31} and P^{32} was not significant.

In the total phosphate figures, one significant increase was shown in roots and one significant decrease in the tops. The soil phosphate was significantly less at 4 μc than at 0.5 μc P^{32} per pot. None of the derived ratios however were affected by P^{32}.

Experiment 3

The data for the first sampling of tomato are tabulated in Table IV. The only significant effect is shown in the soil phosphate, 'tops', at 500 μc P^{32} per pot.

Experiment 3
(1st Sampling)

TABLE IV

Effect of P^{32} levels on dry weights, total P, fertiliser P and soil P, and ratios of these in tomato (mean of 12 pots)

Functions	P^{32} as μc per pot					S.D.
	500	25	4	0.5	0	P = 0.01
Dry weights (g)						
Roots	2.35	2.14	1.97	1.89	2.26	NS
Tops	12.42	11.31	11.26	10.58	11.80	NS
Total P (mg)						
Roots	5.66	5.06	5.10	4.63	5.13	NS
Tops	34.52	30.04	30.96	28.49	29.87	NS
Fertiliser P (mg)						
Roots	0.75	0.87	0.74	0.68	—	NS
Tops	5.84	5.69	5.83	5.30	—	NS
Roots & Tops O . .	8.87	10.45	8.90	8.59	—	NS
Roots & Tops + . .	4.31	3.00	4.24	3.38	—	NS
Soil P (mg)						
Roots	4.92	4.20	4.35	3.95	—	NS
Tops	**28.68**	24.35	25.13	23.19	—	4.86
$\log_e \frac{\text{Fert. P uptake}}{\text{Soil P uptake}} \times 10$						
Roots	2.66	2.96	2.74	2.74	—	NS
Tops	2.94	3.01	3.07	3.03	—	NS
% Fert. P uptake .	21.1	21.4	21.0	20.3	—	NS

O High level of P^{31}
+ Low level of P^{31}
} Mean of 6 pots.

In the second sampling made 3 weeks later, there are no significant changes in any of the functions as shown in Table V, despite the very high fertiliser phosphate uptake which had occurred.

Experiment 3
(2nd Sampling)

TABLE V

Effect of P^{32} levels on dry weights, total P, fertiliser P and soil P and ratios of these in tomato (mean of 12 pots)

Functions	P^{32} as μc per pot					S.D.
	500	25	4	0.5	0	P = 0.01
Dry weights (g)						
Roots	3.34	3.69	3.38	3.32	3.19	NS
Tops	25.55	26.87	24.87	23.87	24.38	NS
Total P (mg)						
Roots	5.46	5.21	4.44	5.11	4.88	NS
Tops	49.31	47.50	46.67	47.46	47.23	NS
Roots & Tops	54.77	52.71	51.11	52.57	52.12	NS
Fertiliser P (mg)						
Roots	0.70	0.63	0.61	△	—	NS
Tops	7.66	8.02	6.99	7.41	—	NS
Soil P (mg)						
Roots	4.76	4.58	4.30	△	—	NS
Tops	42.49	39.49	39.68	40.05	—	NS
$\log_e \frac{\text{Fert. P uptake}}{\text{Soil P uptake}} \times 10$						
Roots	2.60	2.49	2.59	△	—	NS
Tops	2.79	2.86	2.81	2.83	—	NS
% Fert. P uptake	25.7	29.0	24.9	△	—	NS

△ Activities too low for radioassay.
Interaction between P^{31} and P^{32} was not significant.

Experiment 4

The results given in Table VI show that the tracer has had no effect on the dry weights, fertiliser, soil and total phosphate contents in barley grown in a soil high in organic matter (pH 5).

DISCUSSION

In plant nutrition experiments with radioactive isotopes in soil four assumptions are made: (*a*) that the plant tissues do not differentiate between isotopes of the same element when applied in the same chemical form, (*b*) that the degradation products do not affect the plants' metabolism, (*c*) that radioactive material added in the same chemical form as the fertiliser does not enter into isotopic

Experiment 4

TABLE VI

Effect of P^{32} levels on dry weights, total P, fertiliser P and soil P and ratios of these in barley (mean per plant per plot)

Functions	P^{32} as μc per pot						S.D.
	400	125	25	5	1	0	P = 0.01
Dry weights (g)							
Roots	0.091	0.097	0.086	0.080	0.081	0.089	NS
Tops	0.209	0.214	0.200	0.195	0.205	0.189	NS
Roots & Tops	0.300	0.311	0.286	0.275	0.286	0.278	NS
Total P (mg)							
Roots	0.049	0.049	0.047	0.044	0.047	0.048	NS
Tops	0.128	0.125	0.121	0.121	0.118	0.121	NS
Roots & Tops	0.177	0.174	0.167	0.164	0.165	0.169	NS
Fertiliser P (mg)							
Roots	0.067	0.070	0.060	0.065	0.063	—	NS
Tops	0.193	0.189	0.165	0.187	0.179	—	NS
Roots & Tops	0.259	0.259	0.225	0.251	0.242	—	NS
Soil P (mg) (Roots & Tops)	1.37	1.35	1.22	1.26	1.28	—	NS
$\log_e \frac{\text{Fert. P (tops)}}{\text{Fert. P (roots)}}$	1.05	1.00	1.01	1.06	1.04	—	NS
$\log_e \frac{\text{Fert. P uptake}}{\text{Soil P uptake}} \times 100$ (Roots & Tops)	2.92	2.96	2.84	2.97	2.92	—	NS
% Fert. P uptake	3.9	4.1	3.9	3.7	3.6	—	NS

Interaction between P^{31} and P^{32} was not significant.

exchange with non-radioactive soil constituents and (*d*) that radiation effects do not occur, or if they do, they are not of sufficient magnitude to invalidate the results of the experiment.

In nutrition experiments with P^{32} it is likely that assumptions (*a*) and (*b*) are justified. With regard to (*c*) McAuliffe *et al.* [9], have shown that isotopic exchange takes place between solution P^{32} and soil P^{31}. It has been suggested by McAuliffe, Peech and Bradfield [10] that isotopic exchange may effect the conclusions drawn from experiments using P^{32} to determine the uptake of fertiliser phosphate by plants growing in soil. The fertiliser P uptake determined in this way may not be the absolute amount absorbed by the plants, but this is unlikely to invalidate our conclusions in regard

to the effects of radiation on the phosphorus contents of the plants.

This paper is concerned with assumption (*d*). It is well known that small radiation dosages may produce chromosome breaks and cytological changes in living cells (Lea [8]). Although such effects are important in the study of genetics, they may be less important in short term nutrition experiments when the study is confined to one generation of plants.

The nutrition experiments described in this paper are large-scale, well replicated and factorial in design. Thus in each of our experiments from 72 to 144, 6″ pots were employed, using carrier orthophosphate equivalent to 200 and 500 lb. superphosphate per acre labelled respectively with levels of P^{32} varying from 0.5 to 500 μc per pot. Control treatments given carrier phosphate only were included in each experiment.

We determined the effect of treatment on dry weights of tops and roots; total, fertiliser and soil phosphate uptake by tops and roots, and the ratios derived from these values. We define radiation effects as significant changes in any of these values in relation to the control treatments; for dry weights and total phosphate, plants receiving carrier phosphate only, and for the fertiliser and soil phosphate uptake, plants receiving the lowest level of P^{32} were the controls.

The results given in Table II to VI show that radiation effects occurred infrequently. Thus in experiment 1 a 13% increase in the weight of barley tops occurred at the 5 μc P^{32} level but this effect was an isolated one and did not become more pronounced with increasing levels of P^{32}, and at the 400 μc level there was no significant difference compared with control treatment. On the other hand the 400 μc treatment was the only one that had a significant effect on the following ratios: fertiliser P uptake/total P, and fertiliser P uptake/soil P uptake. These sporadic effects are difficult to understand. The fact that they did not become progressively greater with increasing tracer level is probably due to a rapid initial fixation of fertiliser phosphate when added to the soil. Experiments carried out by us show that more than 98% of the fertiliser phosphate was removed from solution within 24 h of its addition to the soil (see appendix B). McAuliffe *et al.* [9]) have shown that when soil is shaken with solutions of P^{31} and P^{32} as orthophosphates, two distinct reactions occur. The first of these, in which the fraction P^{32} solid/P^{32} solution changes logarithmically with time, corresponds to exchange

between phosphate in solution and on the soil surface. Phosphate after sorption on the soil surface undergoes a further reaction, the nature of which is not completely understood. The rapid fixation of P^{32} in soil contrasts with the availability of most of the tracer in sand and water culture experiments.

In all other experiments reported in this paper the dry weights of tops and roots were not affected by the level of P^{32} but small differences in other values occurred occasionally. In no instance however, was fertiliser phosphate uptake affected by tracer level, although we used far higher levels of P^{32} than would normally be used in tracer experiments, and a wider range of specific activities (from 12–29, 760 μc of P^{32} per g P^{31}) than those employed by other workers (see Table IV, Bould *et al.* [4]).

Russell, Adams and Martin [12]) state "... the assumption that radiation from the tracer has not invalidated experimental results can be fully justified only if it has been shown that the rates of absorption of fertiliser and soil phosphorus are unchanged when different activities of tracer are used to label a constant quantity of fertiliser phosphorus". In all our experiments, with one exception, (Experiment 1, 400 μc level of P^{32}), the ratio fertiliser P uptake/soil P uptake was unchanged when various levels of tracer were used to label a constant amount of fertiliser phosphorus.

Thus in using P^{32} in plant nutrition experiments in soil occasional radiation effects may occur. In our experiments these effects have always been less than 15% in magnitude. In many instances no changes occurred that could be attributed to a tracer effect. The possibility of an occasional radiation effect occurring in nutrition experiments with P^{32} makes it imperative to employ a well replicated design containing more than one level of P^{32}, with a good statistical sensitivity to determine whether it has occurred, and, if so, its magnitude. It is unfortunate that full details of experimental design are not always included in published work in which tracer effects are discussed.

If the main treatment effects in experiments which include P^{32} are less than 15% then the interpretation of the data will be difficult in view of possible radiation effects, but in most nutrition experiments differences resulting from treatments are usually much greater than 15%. In precise nutrition experiments however, it is important to know whether radiation effects have occurred and to determine their

magnitude. This can be done by labelling one or more carrier treatments with various tracer levels and determining whether the tracer has had a significant effect on any of the functions discussed previously. If no significant effects can be attributed to the tracer, and should isotopic exchange not affect the interpretation of the results, then it is possible to use P^{32} satisfactorily in soil nutrition experiments, employing the lowest tracer level that is practicable.

SUMMARY

1. Factorial pot experiments with tomato and barley grown in two soils are described in which two levels of carrier phosphate equivalent to 200 and 500 lb. superphosphate per acre were labelled with various activities of P^{32}, from 0.5 to 500 μc per pot containing 2 kg of soil.

2. The dry weights of plant 'tops' and 'roots' were determined as well as total, fertiliser and soil phosphate uptake, and ratios derived from these.

3. It is shown that radiation effects occur at relatively low tracer levels, but that these occur only infrequently. The differences induced between controls and treated plants have in no instance exceeded 15% of the control figures, although the range of specific activity, viz. 12 to 29,760 μc P^{32} per g P^{31} was large. In most plant nutrition experiments in soil the main treatment effect is usually greater than the maximum radiation effects produced in these extensive experiments. The tracer effects, even should they occur, would be unlikely to invalidate the main results.

4. In precise experiments however, it is essential to know whether radiation effects occur and, if so, to determine their magnitude.

5. It is shown that a large proportion of the fertiliser phosphate is immobilised in the soils so that the amount immediately available to the roots is reduced. This may explain the fact that radiation effects did not become progressively more severe with increased tracer activity.

6. The radiation effects in barley and tomato reported in these experiments are not sufficiently great to preclude the use of P^{32} in plant nutrition experiments in soils. It is important however, for investigators to determine under their own experimental conditions whether radiation effects occur in nutrition experiments. This may be of even greater importance when longer-lived isotopes are used in long-term experiments with plants.

ACKNOWLEDGMENTS

We wish to acknowledge with thanks the provision of equipment for radioactive assay by the Agricultural Research Council. We are grateful to the following workers who helped us to harvest the plants: Messrs. J. Tolhurst, H. M. Webber, M. A. Wright and J. Sims. The last named also assisted with radioassay.

Received April 3, 1953.

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APPENDIX

A. *Penetration of P^{32} and Distribution of Roots in Soil*

Soil Penetration Studies. Experiments were carried out to determine the extent of penetration of P^{32} phosphate in soil under conditions similar to those in the main pot experiments. 50 ml of solution 'C' containing 1.3 μc of P^{32} and 42 mg P^{31}, both as orthophosphate, was applied to the surface of the soil in 6″ bitumen painted pots. The pots were watered regularly, and after 11 days the soil was sectioned into 1″ layers, dried, weighed, thoroughly mixed and sub-sampled for analysis.

20 g samples of oven-dry soil were weighed into tall 800 ml Pyrex

beakers and 200 ml of constant-boiling HCl added to each. The beakers were covered with clock glasses, brought to the boil on a hot plate, and gentle boiling continued for 1 hour. The solutions were then filtered through a Whatman No. 50 filter paper into 250 ml graduated flasks, washed with hot HCl and after cooling made up to volume. Aliquots were taken for radio-assay in a M 6 counting tube. The results are as follows:

Layer	P^{32} recovered as a % of the total applied.			
	Pot A		*Pot B*	
0—1″	90.8	95.1	84.7	97.5
1—2″	4.3		12.8	
2—3″	2.9		2.2	

It will be seen that approximately 96% of the P^{32} was retained in the top 1—2″ of soil, hence the reason for standardising the depth of sowing the barley seed in the main experiments.

Distribution of Plant Roots in the Soil. The soil from three pots, similar to those used in the main experiments and each containing one tomato plant (three weeks from transplanting) was divided into 1″ sections. The plant roots were extracted, washed, dried and weighed and the percentage weight of roots in each section determined. The results are given in Table I.

TABLE I

Distribution of tomato roots in soil contained in pots similar to those used in the main experiments

Layer	Root weight per layer as % of total dry wt.			
	Pot A	B	C	Mean
0—2″	81.9	74.2	72.9	76.3
2—3″	9.4	13.9	11.9	11.7
3—4″	4.4	5.2	4.8	4.8
4—5″	4.3	6.7	10.4	7.1

It is clear that the major root concentration was localised in the area containing the largest fraction of P^{32}.

B. *Changes in the water-soluble P^{32} phosphate after addition to soil*

In order to gain some information on the concentration of water-soluble P^{32} to which the roots of tomato seedlings and barley seeds would be exposed after applying radio-active solution to pots, the following experiment was carried out.

353 g samples of fresh soil (moisture content 9.3%) were weighed out into bottles. To each was added, 10 ml of the P^{32} orthophosphate solution containing 0.8 μc of P^{32}, 8.5 and 3.4 mg respectively, P^{31} and 15 ml of distilled water, making a total moisture content of 55 ml. The bottles were allowed to stand at room temperature for the following intervals of time: 5, 15, 30, 45, 60 min and 22 h respectively. One minute prior to the time of extraction 195 ml of distilled water was added to each bottle, which was then shaken

on a reciprocating shaker for 1 min. The contents were transferred rapidly to a Buchner funnel, to which was sealed a No. 50 Whatman filter paper, and suction applied. The filtration was stopped as soon as sufficient filtrate was obtained for counting, viz. about 12 ml, time taken was less than 1 min. One drop of concentrated H_2SO_4 was added to the filtrate to minimise adsorption of phosphate on the glassware. The total volume of water was 250 ml so that the original solution had been diluted 25 times. The results, expressed in counts per min are given in Table II for two solutions having approximately the same radio-activity but different carrier levels.

TABLE II

Effect of time of contact of solution with soil (pH 5.0) on the water-soluble P^{32} orthophosphate at two carrier levels

Time of contact with soil	Counts per min	
	Low P^{31}	High P^{31}
Control — no soil	4186	4000
Soil — 5 min	660	840
,, 15 ,,	440	805
,, 30 ,,	250	540
,, 45 ,,	225	373
,, 60 ,,	221	385
,, 22 h	66	59

The results given in Table II show that there was a very rapid decrease in the water-soluble P^{32} in the first few minutes, probably due to precipitation of calcium phosphate and anion exchange, followed by a slower decrease until after 22 h the counts were reduced to a low level.

This point is of importance in the barley experiment because the P^{32} concentration in the soil solution would be very low when the first roots were formed.

C. *Absorption of P^{32} by barley seeds in soil*

Information was required on the absorption of P^{32} by barley seeds from soil one and two days after seeding. The following pot experiment was carried out to elucidate this point. Four 6″ pots containing soil were sown with barley, 9 seeds per pot, on the following dates:

2 pots sown at 4 p.m. on December 19th.
2 ,, ,, ,, 4 ,, ,, December 20th.

At 4.15 p.m. on December 20th each pot received 50 ml of radioactive solution containing 1.2 μc P^{32} and 42 mg P^{31}, both as orthophosphate. At 4 p.m. December 21st the seeds were removed from the soil, washed twice with acidified phosphate, then dilute acid and finally distilled water. They were digested with mixed acids, made up to constant volume and radio-assayed. In all cases the counts were negligible showing that absorption by the seed under these conditions, during the first two days, was very small, and by this time most of the P^{32} was not water-soluble.

AN ANALYSIS OF THE ACID SOIL COMPLEX BY THE USE OF INDICATOR PLANTS

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INTRODUCTION

The deleterious effects of soil acidity on crop growth have recently been reviewed by H e w i t t (1952) [1]). With the aid of sand cultures H e w i t t (1948) [2]) has studied the visual symptoms in plants grown in pot sand cultures simulating conditions in acid soils. Concurrently with this work at Long Ashton, field trials were started during 1948 on acid soils of differing parent material, to observe, under field conditions, the crops studied in sand culture.

METHODS

Four experimental Centres 1–4, were established during 1948. Centres 1–2 were situated on soils derived from the Old Red Sandstone and Centres 3–4 on soils from granite. Relevant data for the Centres are given in Table I.

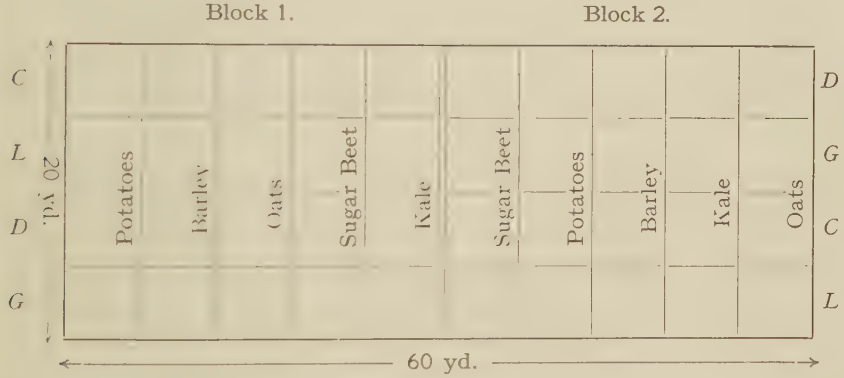
TABLE I

Centres 1–4					
Centre	County	Parent material of soil	Field culture prior to 1948	Site altitude	Mean annual rainfall (inches)
1	Somerset	Old Red Sandstone	Permanent pasture	200 ft.	35
2	Somerset	Old Red Sandstone	Old arable	200 ft.	35
3	Devon	Granite	Permanent pasture	900 ft.	42
4	Devon	Granite	Permanent pasture	900 ft.	42

Conditions for growth were good at the two Centres in Somerset; the two in Devon, being on the edge of the high granite plateau of Dartmoor, were handicapped by a late and short growing season.

There was twofold replication of treatments at each site. The design adopted (Fig. 1) had two blocks, each divided longitudinally into five strips for the indicator crops with the four experimental treatments applied transversely.

On the old arable site at Centre 2, the amount of organic matter was considerably less than at the other Centres, and the deleterious effects of the acid conditions were greater.



Treatments: *C:* N.P.K.
L: N.P.K. plus ground limestone
D: N.P.K. plus ground dolomite
G: N.P.K. plus gypsum.

Fig. 1. Plan of experimental plots.

During the spring of 1948, limestone, dolomite and gypsum were each applied at 3 tons per acre, and were not applied again during the period of the experiment (1948-51). Applications of N.P.K. (6 cwt. ammonium phosphate and 2 cwt. muriate of potash) were made to all plots in 1948, 1949 at the rate of 8 cwt. per acre. Potash was omitted from the mixture in 1950.

The plots were ploughed each year and the usual cultivations carried out to obtain friable seed beds.

The mean soil reactions of the various treatments at each Centre in October 1948 are shown in Table II.

TABLE II

Treatment	Soil pH at Centres 1–4 (Oct. 1948)			
	Old Red Sandstone		Granite	
	Centre 1	Centre 2	Centre 3	Centre 4
C	5.0	4.4	5.1	4.9
L	6.6	5.8	6.0	6.1
D	6.1	5.8	6.0	6.1
G	4.6	4.3	4.7	4.8

The following indicator crops were used for the 3 year period:

Oat	var.: Star
Barley	var.: Spratt Archer
Kale (*Brassica oleracea acephala*)	var.: Marrow Stem
Potato	var.: Majestic
Sugar beet	var.: Sharpe's Klein E.

After the first year the crops were rotated over the strips.

RESULTS

Observations were made during the growing season of symptoms on leaf and root, habit of growth and vigour. Leaf samples were taken once during each season and analysed quantitatively for Ca, P, K, Mg and Mn. Yields were recorded at the usual seasonal times.

Symptoms

The symptoms associated with each crop and treatment are listed in Table III. Diagnosis was made according to the visual technique of Wallace (1951) [3]).

Centre 1, where the most productive crops were obtained, showed slight Mg deficiency in barley and oat. Barley and sugar beet showed Al toxicity, more so in the second and third years, as the old turf gradually disappeared. At Centre 2, the acid soil complex produced crop failures associated with deficiencies of Mg and Mo and toxicities of Al and Mn. Crops at Centres 3–4, showed an almost identical syndrome with Mg deficiency and Al toxicity. There were no symptoms of Mn toxicity. The occurrence of N deficiency at Centre 4, was thought to be due to the decomposing remains of bracken rhizomes.

The carbonates, in general, eliminated the effects of Al and Mn toxicities but induced B deficiency in sugar beet. Gypsum induced or accentuated Mg deficiency; reduced the severity of Mn toxicity, but failed to correct Al toxicity. At the bottom of Table III the

TABLE III

Diagnosis by visual symptoms of mineral deficiencies and excesses																
	Centre 1				Centre 2				Centre 3				Centre 4			
	C	*L*	*D*	*G*	*C*	*L*	*D*	*G*	*C*	*L*	*D*	*G*	*C*	*L*	*D*	*G*
Oat				—Mg	—Mg +Mn +Al			—Mg +Al				—Mg				—Mg
Barley	+Al			—Mg +Al	—Mg +Al			—Mg +Al	+Al			+Al	+Al			+Al
Kale					—Mo +Mn +Al			—Mg +Al					—N	—N	—N	—N
Potato					—Mg +Mn			—Mg								
Sugar beet	+Al			+Al	+Mn +Al	—B	— B	+Al	+Al	— B	—B	+Al	+Al	—B	—B	+Al
Syndrome of acid soil	—Mg +Al				—Mg	—Mo	+Mn	+Al	—Mg +Al				—N	—Mg	+Al	
Syndrome after liming					—B				—B				—N	— B		

visual syndromes, given by all crops, are summarised for each Centre.

Aspects of the different mineral disorders are as follows:

Deficiencies: As N, together with P and K, was supplied annually to the plots it was not expected that this element would be deficient, but N deficiency appeared, particularly in kale, at Centre 4.

Mg deficiency occurred at Centre 2 on the Control plots but was also evident at all Centres on the gypsum treatment. Over the three year period this influence of gypsum was more striking towards the end than at the beginning. Oat and kale were most affected by Mg deficiency which killed some of the oats during a dry spell in 1949. Whilst potato and barley showed specific symptoms of Mg deficiency, yield was not affected.

Symptoms of Mo deficiency, partially masked by Mn toxicity, were apparent only at Centre 2. In more recent experiments of the writer at Centres 1–2, the diagnosis of Mo deficiency has been confirmed by the occurrence of whiptail symptoms in cauliflower plants.

One effect of adding limestone or dolomite at Centres 2, 3 and 4, was to induce B deficiency in sugar beet. As the symptoms only occurred towards the end of the growing season, the effect on yield was not appreciable. No boron deficiency occurred at Centre 1.

Toxicities: Symptoms of acute Mn toxicity on the leaf and retardation of growth occurred chiefly at Centre 2. In kale the symptoms persisted throughout the growing period (Plate I), whereas in cereals, symptoms occurred during the early stages of growth, but eventually disappeared. The potato also showed symptoms of Mn excess which were more pronounced in a dry summer

Al toxicity was evidenced by symptoms in the root system. The syndrome of Al toxicity, which has been described by Hewitt (1948) [2]), was observed at all Centres on barley and sugar beet. This toxicity induced a syndrome similar to phosphate deficiency, and the only means of detecting the difference in the field was by the examination of root systems.

Multiple disorders: Table III shows that in kale (at Centre 2) there was a combination of nutrient disorders on one crop. Plate II illustrates the necrotic spots due to Mn toxicity and the chlorotic marbling due to Mg deficiency.

The synergistic action between Mn and Al excesses as described by Hewitt (1952) [1]) was apparent at Centre 2. It was particularly

Plate I. Manganese toxicity in marrowstem kale. Note cupping of leaves and necrotic spots towards leaf edges.

Plate II. Magnesium deficiency (chlorosis) and manganese toxicity (necrotic spotting) in marrowstem kale.

noticeable on the Control. This effect was less striking on crops in the gypsum treatment, as the Mn concentration in the plant tissues was considerably reduced compared with the amount found in crops on the Controls.

Yields

Table IV includes the mean yields for all Centres for each crop. The individual yields obtained at each Centre are given in the histograms (Figs. 2–2*d*).

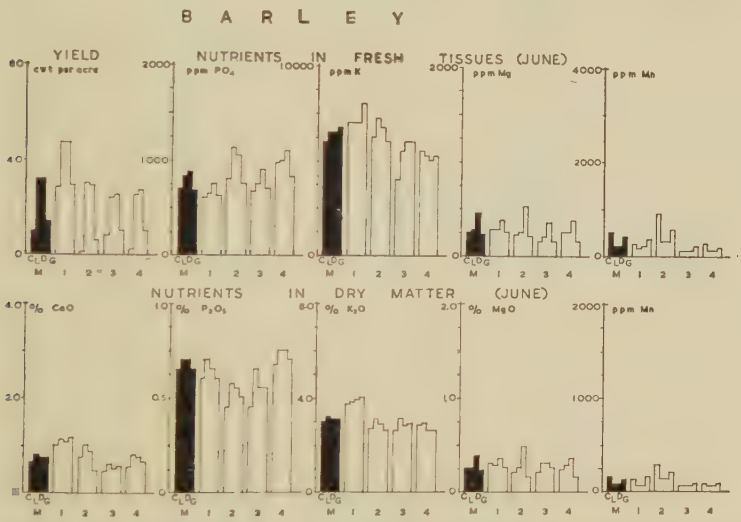


Fig. 2–2*d*. The yields, and P, K, Ca, Mg and Mn leaf contents of the 5 indicator crops. The tissue tests and dry matter estimations were carried out from the same leaf samples.

M: Mean of Centres 1–4. (1948–50)

Old Red Sandstone { *1:* Mean of Centre 1. (1948–50); *2:* Mean of Centre 2. (1948–50)

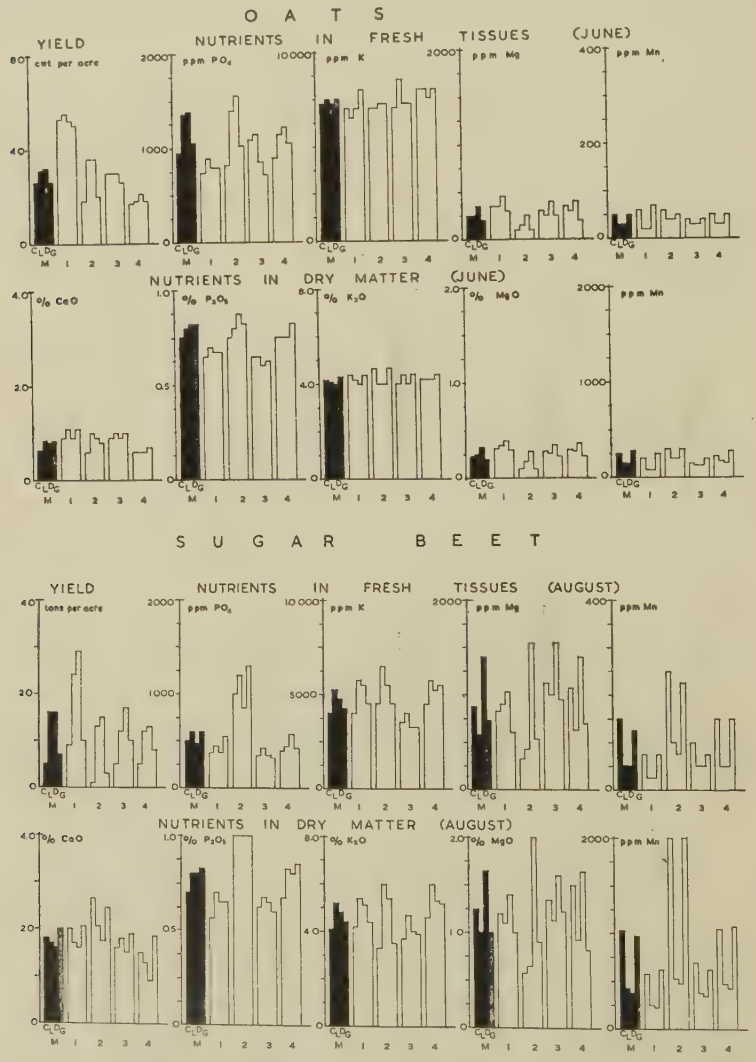
Granite { *3:* Mean of Centre 3. (1948–50); *4:* Mean of Centre 4. (1948–50)

C: N.P.K. *L:* N.P.K. plus limestone

D: N.P.K. plus dolimite *G:* N.P.K. plus gypsum.

The overall yields show that barley and sugar beet responded strikingly to a rise in soil pH where the carbonates had been applied; oats and kale showed a limited increase, whilst potatoes made only a small gain. The addition of gypsum gave no significant yield response.

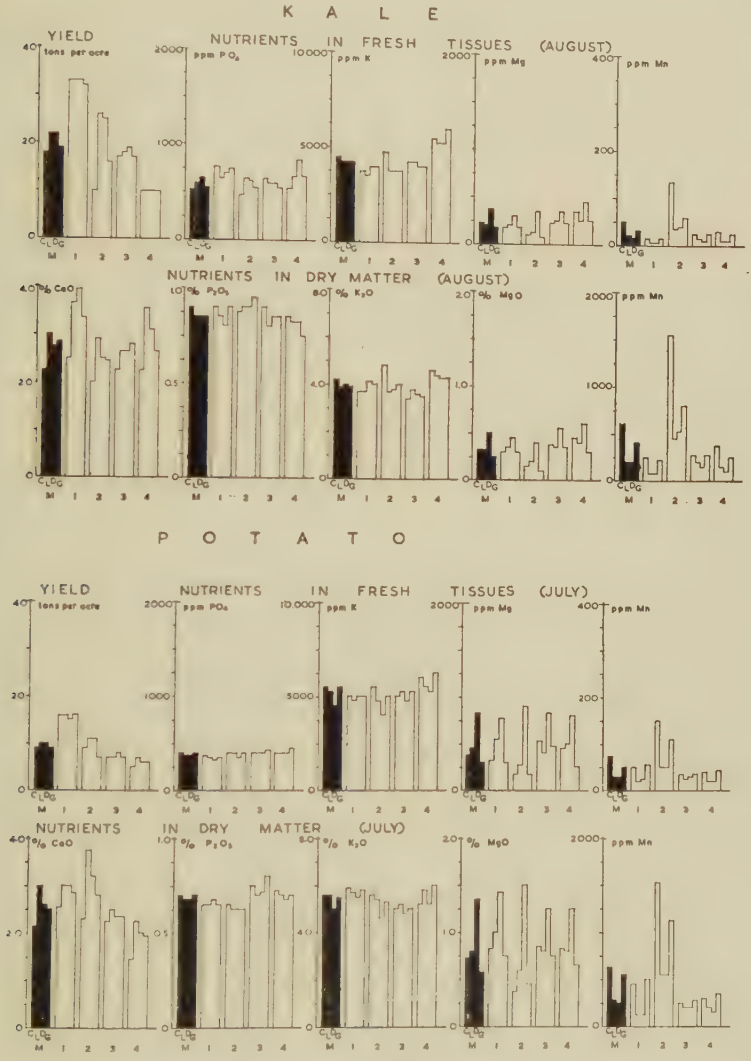
At the individual Centres, barley and sugar beet yields showed a significant increase where limestone and dolomite had been applied. A slight improvement in yield occurred in these crops where gypsum



had been applied, at Centres 3 and 4. At Centre 2, oats, kale and potato showed substantial responses to the carbonate treatments.

The histograms show that the Somerset Centres greatly outyielded the Devon Centres. Excluding any nutritional effects this can be

largely attributed to the better growing conditions at Centres 1 and 2 in Somerset (see Table I).



Chemical data

One leaf sample was collected from each crop in order to determine the amounts of soluble P, K, Mg and Mn in tissue extracts from fresh material, and the total contents of the same elements together with Ca in leaf dry matter.

TABLE IV

Means of yields and chemical data for Centres 1–4

Crop	Treatment	Yield	Tissue Tests p.p.m. Fresh wt.				Dry Matter analyses % Dry wt.				p.p.m.
			PO_4	K	Mg	Mn	CaO	P_2O_5	K_2O	MgO	Mn
Oat	*C*	26 cwt.	945	7333	257	47	0.68	0.76	4.22	0.23	234
	L	31 ,,	1362	7425	244	32	0.84	0.80	4.07	0.25	158
	D	32 ,,	1380	7308	334	31	0.78	0.83	3.98	0.33	156
	G	26 ,,	1052	7500	206	51	0.86	0.79	4.35	0.20	273
		± 3.29									
Barley	*C*	10 cwt.	694	6175	264	50	0.65	0.63	3.00	0.24	135
	L	32 ,,	837	6558	267	21	0.81	0.71	3.23	0.26	71
	D	32 ,,	869	6571	457	21	0.73	0.70	3.08	0.37	65
	G	14 ,,	674	6716	226	39	0.74	0.64	3.09	0.20	126
		± 7.18									
Kale	*C*	18 tons	522	4521	216	51	2.24	0.89	4.21	0.33	602
	L	22 ,,	595	4225	209	18	3.01	0.85	3.94	0.33	213
	D	22 ,,	648	4187	372	17	2.78	0.84	3.96	0.50	216
	G	19 ,,	551	4278	182	30	2.84	0.86	3.95	0.26	393
		± 1.81									
Potato	*C*	9 tons	394	5420	378	72	2.13	0.70	5.56	0.72	636
	L	10 ,,	381	5175	442	29	2.91	0.67	5.59	0.80	270
	D	10 ,,	378	4787	827	29	2.61	0.68	5.00	1.36	254
	G	9 ,,	404	5400	303	49	2.52	0.69	5.55	0.58	562
		± 0.44									
Sugar beet	*C*	5 tons	491	4147	871	139	1.79	0.71	4.12	1.23	1015
	L	16 ,,	597	5290	577	61	1.72	0.81	5.23	1.01	421
	D	16 ,,	474	4681	1399	52	1.56	0.81	4.84	1.66	375
	G	7 ,,	607	4333	731	126	1.97	0.83	4.39	1.03	978
		± 3.06									

The overall means for these elements are given in Table IV. The histograms (Figs. 2–2*d*) show the means (1948–51) for each Centre together with the overall mean for comparative purposes. In the histograms the values for nutrients P, K, Mg and Mn in tissue extracts and in dry matter are placed vertically adjacent.

The relationships between symptoms, yield and chemical data are discussed below for each crop. The values of nutrient contents are based on the dry matter estimations.

Oat

Although there are differences in leaf contents of P no oat plants in any treatment were deficient in this element. In fact, the highest yielding Centre (1) had the lowest P content in tissue extracts. The K content was satisfactory in all treatments. At Centre 2, where symptoms of Mg deficiency were pronounced, the Mg content was low (0.10% MgO) and this deficiency doubtlessly caused the reduced yields. The Mn level never exceeded 320 p.p.m. and did not appear to affect growth.

Barley

The contents of P, K and Mg in the barley leaves were satisfactory in all treatments and at all Centres. The leaf content of Mn was high (275 p.p.m.) only at Centre 2 on the Control treatment. Although Table III indicates that Al reduced the growth of barley, there is no chemical evidence of accumulation of Al in these barley plants. McLean and Gilbert (1927) [4]) showed that Al could not be detected in barley leaves. The alternative of estimating Al in the root was not attempted because of soil contamination.

Kale

Of the five indicator crops, kale shows the best agreement between symptoms, yield and chemical data.

The leaf content of P, K and Ca was high at all Centres. The effect of N deficiency was marked at Centre 4 (see Table III). At Centre 2, where symptoms of Mg deficiency and Mn toxicity were so apparent, the Mg content of the leaf, particularly on the gypsum treatment, was very low (0.10%MgO). The Mn content in the control leaves was 1550 p.p.m. but where gypsum had been applied it fell to 800 p.p.m. Mn in the kale at the Devon Centres did not exceed 375 p.p.m.

The relation between Mo deficiency symptoms and the leaf con-

tent of Mo in the presence of Mn excess is shown in Table V for the year 1949 (Centres 2 and 3).

It can be seen that whilst symptoms of Mo deficiency and Mn toxicity occurred at Centre 2, there were no symptoms at Centre 3. Centres 1 and 4, not listed in Table III, were similar in all respects to Centre 3. The accumulation of Mn in the kale leaves at Centre 2 (1730 p.p.m.) produced symptoms which masked the syndrome of Mo deficiency in the later stages of growth.

TABLE V

The Mo and Mn content of marrowstem kale at Centres 2 and 3. (Leaves sampled 10.8.49)

	Treatment	Symptoms	Mo p.p.m. D.M.	Mn p.p.m. D.M.
Centre 2	*C*	severe +Mn trace —Mo	0.10	1730
(Old Red	*L*	none	0.84	467
Sandstone)	*D*	none	0.68	577
	G	trace +Mn	0.10	1075
Centre 3	*C*	none	0.23	350
(Granite)	*L*	none	0.52	225
	D	none	0.63	142
	G	none	0.16	305

Potato

The contents of P, K and Ca were satisfactory in potato leaves on all treatments. The Mg content was low at Centre 2 in Control and gypsum treatments, whilst Mn was high (1500 p.p.m.) on the Control. The effect of gypsum again was to reduce the Mn content to 1100 p.p.m.

Sugar beet

Al toxicity on sugar beet caused crop failures similar to those found with barley. Although the yields were lowest on the Control and gypsum treatments the P leaf contents of the plants growing on these treatments were higher than on the other treatments. The accumulation of Mn (2000 p.p.m.) at Centre 2 was not associated with any specific symptom.

As Al toxicity was considered to be the limiting factor to growth, the content of Al in the root was estimated. The results are given in Table VI.

TABLE VI

The content of P and Al in sugar beet roots. Mean for all Centres (1948)		
Treatment	% P_2O_5 on D.M.	p.p.m. Al in D.M.
C	0.19	11.5
L	0.27	8.5
D	0.25	10.7
G	0.24	12.1
	± 0.025	± 1.78

They show that there was no marked difference between the Al content of the roots due to the treatments.

Wright (1943) [5]) noted that P accumulates in sugar beet roots growing in a media with a high Al. There is no evidence, however, in Table VI that P accumulated in the Control and gypsum treatments. Extracts from the root and leaf growing on Control and limestone treatments were examined chromatographically but no differences in Al contents were found.

DISCUSSION

Attempts were made to assess some of the deleterious factors in acid soils by using suitable indicator crops in combination with soil treatments designed to affect the pH, calcium and magnesium status of the soils. As all plots received an annual N.P.K. fertiliser the parts played by these three elements could not be determined.

The results showed that on the plots receiving no special treatment deficiencies of Mg and Mo and excesses of Mn and Al, produced deleterious effects although these factors did not all produce measurable effects at all Centres.

The effect of the two liming treatments given — ground limestone and ground dolomite — was to overcome the effects of excess Mn and Al in addition to correcting the deficiency of Mg. These treatments also induced boron deficiency at three centres.

Gypsum did not produce the good effects of the liming treatments. It reduced the manganese contents of the crops but induced a deficiency of Mg in some crops.

The experiments have thus shown the complexity of acid soil effects and the differences that may occur between soils.

SUMMARY

(1) Mineral deficiencies and excesses were recorded visually in oat, barley, kale, potato and sugar beet crops used as indicator crops, grown on acid soils derived from the Old Red Sandstone (Centres 1–2) and Granite formations (Centres 3–4). Four treatments were given (*a*) N.P.K. (Control), (*b*) N.P.K. plus limestone, (*c*) N.P.K. plus dolomite, and (*d*) N.P.K. plus gypsum. Yields at harvest and chemical data of leaf analysis are presented.

(2) Centres 1–2 showed, in treatments (*a*) and (*d*), deficiencies of Mg and Mo and excesses of Al and Mn; where the two liming materials were applied B deficiency occurred in sugar beet. At Centres 3–4 on the granite, treatments (*a*) and (*d*) showed only Mg deficiency and Al excess; B deficiency also occurred after the application of carbonates. No Ca deficiency was recorded at any Centre.

(3) At all Centres, limestone and dolomite significantly increased yields above (*a*) N.P.K. and (*d*) N.P.K. plus gypsum. Treatments (*a*) and (*d*) showed similar low yields.

(4) The limestone and dolomite increased leaf content of Mg and Mo, and decreased amount of Mn. Gypsum decreased leaf content of Mn and Mg; Mn excess symptoms were eliminated and those of Mg deficiency accentuated.

(5) Although the symptoms of Mn excess were confirmed by large amounts of Mn in the leaves of susceptible crops, Al toxicity was not accompanied by an accumulation of Al in sugar beet which, along with barley, appeared most susceptible to this element.

(6) Limitations of these acid soils for crop production can be attributed in part to mineral deficiencies and excesses occurring singly or together. The mineral status was related to the parent material of the soils.

ACKNOWLEDGEMENTS

This work was carried out under grants by the Agricultural Research Council to whom grateful acknowledgement is made.

My thanks are particularly due to Mr. J. Redmond, for all the chemical determinations, to Mr. G. H. Jones for the photographs, to Dr. D. A. Boyd, for statistical assistance and lastly to the farmers who helped in every way possible.

Received May 20, 1953.

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Plant and Soil V, no 1 October 1953

SOME EFFECTS OF HEAVY METALS ON PLANTS GROWN IN SOIL CULTURE

PART I. THE EFFECT OF COBALT ON FERTILISER AND SOIL PHOSPHATE UPTAKES AND THE IRON AND COBALT STATUS OF TOMATO

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INTRODUCTION

The effects of excessive dosages of heavy metals in producing iron deficiency symptoms in susceptible crops are well known in water culture (Somers and Shive [19]), Millikan [11] [12] [13]), Warington [23])); sand culture (Hewitt [7])), soil culture (Nicholas [15] [16]), Hunter and Vergnano [8])) as well as in the field (Wallace and Hewitt [21])). The way in which iron deficiency symptoms are induced by toxic amounts of metals is less clear.

In a soil culture experiment with tomato described in this paper, the effect of cobalt excess on fertiliser and soil phosphate contents was examined on two occasions during one season. Dry weight yields and iron and cobalt contents were also determined in tops and roots. Iron deficiency symptoms accompanied by retardation of growth were produced at both harvests in the cobalt treated plants, and were pronounced at the higher cobalt level. These effects were less marked at the higher fertiliser phosphate level, in marked contrast to the usual effect of high phosphate in inducing iron deficiency. The total and fertiliser phosphate contents of the roots and tops decreased with increased cobalt treatments at both samplings. The data confirm that the total iron in tomato as μg per g dry weight is not correlated with symptoms of iron deficiency.

EXPERIMENTAL

The soil used in the factorial experiment was derived from New Red Sandstone and was taken from the NK (omit P) treatment under grass in a

long-term manurial experiment with fruit trees at Long Ashton. The soil, which was air dried, thoroughly mixed and sieved, had a pH of 6.5. 1.8 kg of soil was weighed into each of 228 bitumen painted 6″ clay pots. The pots were arranged in 6 blocks, with 18 treatments per block, incorporating 3 levels of cobalt at 0, 1 and 4 milliequivalents (meq.) per pot (Co_0, Co_1, Co_4), two levels of P^{31} as $NaH_2P^{31}O_4$ equivalent to 500 and 1,000 lb. superphosphate per acre (P_1^{31}, P_2^{31}) and 3 levels of P^{32}, also as orthophosphate, at 0, 2 and 4 μC per pot, (P_0^{32}, P_2^{32}, P_4^{32}). The effect of adding Mo at 5 and 10 meq. was tested in an extra replicate at the Co_4, P_2^{31}, P_4^{32} level. Two replicate treatments per block were adjacent thus providing a 'split plot' design based on two samplings. Tomato seed *var.* Market King was sown in a standard compost and the plants were transplanted (1 plant per pot) into 6″ pots containing the test soil, a month after seeding. Care was taken to remove the compost from the roots of the plants before transplanting. The following supplement was given in solution to each pot: K_2SO_4 0.28 g, $MgSO_4.7H_2O$ 0.14 g, $NaNO_3$ 0.7 g. A week after planting when the plants were established the cobalt treatments at 0, 1 and 4 meq. were added (50 ml per pot) followed by the radioactive sodium dihydrogen phosphate at the two rates.

Sampling Procedure

The plants were harvested 3 and 6 weeks after the application of the tracer phosphate. They were subdivided into tops and roots, dried in beakers and weighed. After weighing, the two replicates were bulked, for all subsequent determinations. Full details of the root washing have been given elsewhere (Bould, Nicholas and Thomas[3]).

Chemical Methods

The tops and roots respectively were digested with nitric and perchloric acids. Care was taken to deal with the samples in ascending order of radioactivity thus avoiding contamination of the samples. Total phosphorus was determined by the vanadate method (Hanson[4]), iron by α, α-dipyridyl (Moss and Mellon[14]) and cobalt by nitroso-R-salt (Welcher[24]).

Radioassay

The assay of plant digests was made by solution counting in a M.6 tube as described by Veall[20]). Details of the computation of the fertiliser and soil phosphorus uptake by the roots and tops respectively have been given in a previous paper (Bould *et al.*[3]).

The following notation is used in this paper Co_0, Co_1, Co_4, indicating milliequivalent levels of the metal applied per pot; P_1^{31}, P_2^{31} the two rates of fertiliser phosphate used, equivalent to 500 and 1,000 lb. superphosphate per acre, and P_0^{32}, P_2^{32}, P_4^{32}, the μc levels of tracer phosphate added per pot.

A

B

Plate I. A. Cobalt induced iron deficiency.
B. Cobalt injury, necrotic areas on stems and petioles.

A

Co_0 Co_1 Co_4

B

Co_0 Co_1 Co_4

Plate II A. Fertiliser phosphate equivalent to 500 lb. superphosphate per acre.
B. Fertiliser phosphate equivalent to 1000 lb. superphosphate per acre.
Effect of increasing Co at each phosphate level.
Note injurious effects of Co are not so marked at the higher phosphate level.

RESULTS

Visual Symptoms

Symptoms of iron deficiency appeared first in the Co_4 treatments about a week after the application of the metal (Plate IA). They became progressively worse and the plants remained stunted and necrotic areas developed on the stems and petioles (Plate IB). In the Co_1 treatments only slight iron chlorosis was observed. There was a marked difference in the vigour of plants grown at the two phosphate levels at each cobalt treatment and the injurious effects of Co were not so marked at the higher phosphate level (Plate II). Phosphate deficiency appeared in plants given the lower fertiliser phosphate treatment towards the end of the experiment. The addition of molybdenum at 5 or 10 meq. per pot did not alleviate the injurious effects of Co_4; the plants remained stunted and chlorotic.

Dry Weights

The statistical analysis of the dry weights of tomato (roots and tops respectively) for the 1st and 2nd harvests are given in Table I.

TABLE I

Dry weights (g) of roots and tops of tomato (Mean of 54 values)				
Fertiliser Phosphate Levels	1st Sampling		2nd Sampling	
	Roots	Tops	Roots	Tops
$P^{81}{}_1$	0.24	1.06	0.48	2.18
$P^{81}{}_2$	0.27	1.38	0.78	4.08
P	0.05	0.001	0.001	0.001
S.D.	0.02	0.17	0.05	1.1

The dry weights are significantly increased in roots and tops at the higher level of fertiliser phosphate. At the 1st sampling the increase in the weight of tops resulting from fertiliser phosphate treatment is greater ($P = 0.001$) than that for the roots ($P = 0.05$). This indicates ready translocation of phosphate from the roots during the early stage resulting in increased growth of the tops. By the 2nd sampling, the roots had fully developed, and the effect of fertiliser phosphate on the yields of roots and tops was similar ($P = 0.001$).

The effects of cobalt on the dry weights of tops and roots are shown in Table II.

TABLE II

The effect of Co on the dry weights (g) of roots and tops respectively (Mean of 36 values)

meq. Cobalt added to the soil	1st Sampling			2nd Sampling	
	Roots	Tops		Roots	Tops
Co_0	0.28	1.43		0.69	3.56
Co_1	0.28	1.28		0.72	3.51
Co_4	0.20	0.95		0.47	2.40
P	0.001	0.01	0.001	0.001	0.05
S.D.	0.04	0.16	0.21	0.06	0.90

Dry matter in roots is significantly depressed 0.001 level) by Co_4 at both harvests. The effect of cobalt on the dry matter in 'tops' was similar but less marked, especially at the 2nd harvest.

Chemical Data

The total and fertiliser P contents were determined in roots and tops respectively and these values were also expressed on a dry matter concentration basis. Soil P was calculated as the difference between total P content of the tops and roots combined and the sum of fertiliser P content (at harvest) and the P content of the plants at the time of adding the radioactive solution to the test soil. The twelve plants analysed for P content at this stage were of the same age as those used in the main experiment. The ratio fertiliser P/soil P contents was determined at the two samplings as a check on possible P^{32} effect on the plants [3]). The total Co and Fe contents of tops and roots, as well as their concentrations in dry matter were determined. A summary of the main statistical effects is available for inspection at Long Ashton.

1. *Total, Fertiliser and Soil Phosphate Status*

(*a*) Effect of Fertiliser Phosphate Treatment. The statistical data show that the total P and fertiliser P contents in roots, tops, and roots and tops combined were significantly increased at the higher level of fertiliser P application. At the 1st sampling, soil P uptake was unaffected by fertiliser P treatment. At the 2nd sampling plants receiving the higher P level had a significantly greater soil P content than those at the lower fertiliser P treatment. When soil P is expressed on a dry weight basis, there was a greater concentration of soil P in the plants receiving the lower fertiliser P treatments at both harvests.

(*b*) Effect of Cobalt Treatment. The effects of the cobalt treatments on the phosphate status of roots and tops at the two samplings are given in fig. 1A and B. The highest Co treatment significantly depressed the total and fertiliser P contents in roots and tops respectively at both samplings, presumably due to a similar reduction in dry weight yields of the plants. Total P expressed on a concentration basis in dry matter was depressed in the roots at the 1st sampling at Co_4 only; there was no effect at the 2nd sampling. In

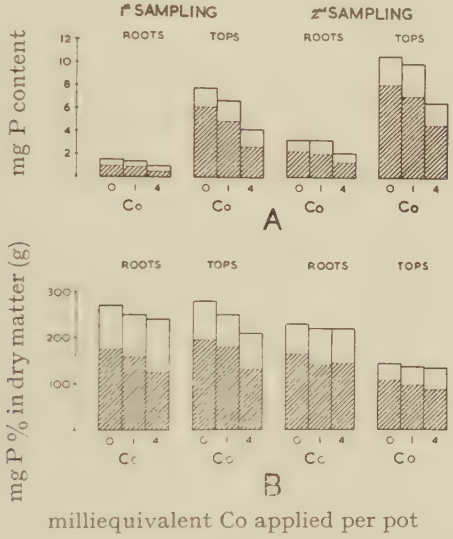


Fig. 1. The effects of cobalt on total P and fertiliser P contents of roots and tops of tomato at two harvests.

▨ Fertiliser P
□ Soil P (by difference)

the tops at 1st sampling there was a significant reduction in total P (as mg P % in dry matter) with increasing Co application. At the 2nd sampling however there was only a slight reduction at the Co_4 level only. Fertiliser P content (as mg or mg % P in dry matter) was consistently depressed at the highest Co treatment in roots and tops at both harvests. The effects of Co treatments on the uptake of soil P (mg and mg % P in dry matter) are shown in Table III.

At the 1st harvest soil P content is depressed with increased Co level whereas at the later sampling the Co_4 rate only has depressed

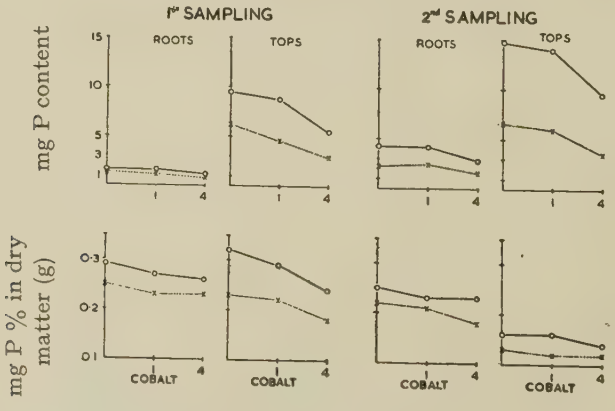
TABLE III

The effects of Co treatments on the soil P content of tomato (roots and tops combined). (Mean of 12 values)

Meq. Co added to the soil	1st Sampling		2nd Sampling			
	soil P (mg)	% soil P (mg) in dry matter (g)	soil P (mg)		% soil P (mg) in dry matter (g)	
Co_0	2.05	56.7	3.17		39.3	
Co_1	1.81	59.0	3.57		44.0	
Co_4	1.33	59.2	2.69		47.8	
P	0.001	N.S.	0.01	0.05	0.01	0.05
S.D.	0.13		0.60	0.44	6.3	4.6

it. When this fraction is expressed as % soil P (mg) in dry matter (g), Co has had no effect at the 1st harvest but at the 2nd sampling the Co_4 treatment resulted in a higher concentration of soil P in the plants.

Fig. 1A and Table II demonstrate that the effect of Co on the total and fertiliser P contents of roots and tops is closely paralleled by the depressing effect of cobalt on the dry weight yields of the plants. In fig. 1B all the phosphate results expressed as mg % P in



milliequivalent Co applied per pot

Fig. 2. The effects of cobalt and fertiliser phosphate treatments on the total P contents of roots and tops of tomato.

x - - - - - x fertiliser phosphate applied equivalent to 500 lb. superphosphate per acre

o ——— o fertiliser phosphate applied equivalent to 1000 lb. superphosphate per acre

dry weight are smaller at the 2nd than the 1st harvest, especially in the tops.

(*c*) Interaction of Cobalt and Fertiliser Phosphate Treatments. Figs. 2, 3 and 4 illustrate the interaction between the different rates of Co and P treatments on total P, fertiliser P and soil P respectively.

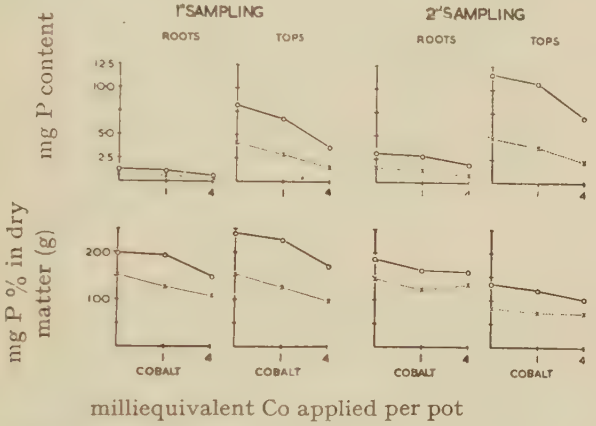


Fig. 3. The effects of cobalt and fertiliser phosphate treatments on the fertiliser P contents of roots and tops of tomato.

x - - - - - x fertiliser phosphate applied equivalent to 500 lb. superphosphate per acre

o ——— o fertiliser phosphate applied equivalent to 1000 lb. superphosphate per acre

The depressing effect of Co, especially at the Co_4 level, on the total P content, fertiliser P uptake of roots and tops is illustrated. When expressed on a dry matter concentration basis the plants receiving the lower phosphate treatment contained more soil P than those given the higher phosphate level.

2. *Cobalt and Iron Status*

Although the roots were thoroughly washed and the sandy soil was easily removed from them it is likely that the Co and Fe contents of the roots may be too high because of soil contamination.

(*a*) Effect of Fertiliser Phosphate Treatments. At the 1st sampling fertiliser P treatments had no effect on the Co and Fe contents of the roots or tops despite the increased dry weight yields of tops ($P = 0.001$) and roots ($P = 0.05$) resulting

from phosphate treatments. At the 2nd sampling the total Co in both roots and tops and Fe (tops only) increased at the higher fertiliser P level. When the metal contents are expressed in p.p.m. dry matter, however, fertiliser P was without effect.

(*b*) Effect of Cobalt Treatments. The Co contents of the roots and tops of plants not treated with the metal were often

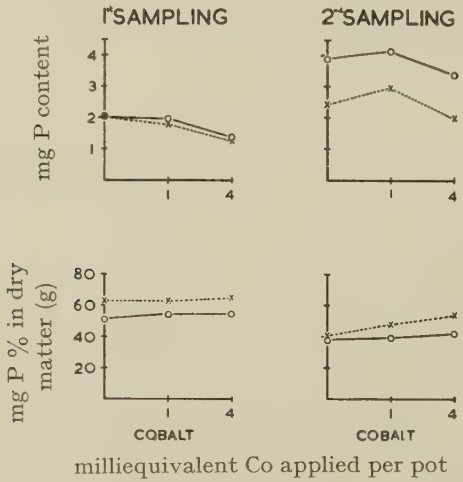


Fig. 4. The effects of cobalt and fertiliser phosphate treatments on the soil P contents of tomato (whole plants).

x - - - - - x fertiliser phosphate applied equivalent to 500 lb. superphosphate per acre

o ——— o fertiliser phosphate applied equivalent to 1000 lb. superphosphate per acre

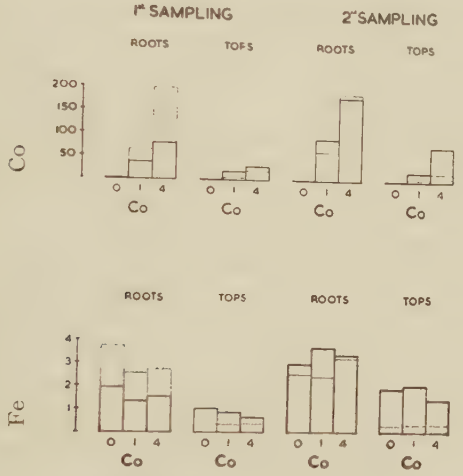
below the limits of chemical detection. Fig. 5 illustrates the effects of Co treatments on the Co and Fe status (as mg Fe, μg Co and mg Fe or μg Co per g dry weight). The Co status of the roots and tops respectively reflected the Co treatments at both harvests. The Co and Fe contents were greater in the roots than the tops but these differences may have resulted from soil contamination. The metal contents were greater in the plant parts at the 2nd harvest. At the 1st sampling the Co_4 treatment depressed the total Fe content of the tops only. When Fe values were expressed in mg per g dry weight there were no effects due to cobalt.

(*c*) Interaction of Cobalt and Phosphate

Treatments. The cobalt treatments were reflected at both phosphate levels.

3. *Effect of Phosphorus-32*

In a previous paper (Bould *et al.* [3]) it was recommended that each carrier level should be labelled with two tracer levels to check whether the tracer had affected the plants during the experiments.



milliequivalents Co applied per pot

Fig. 5. The effect of cobalt treatment on cobalt and iron content of roots and tops of tomato.

——— total Fe in mg or total Co in μg

- - - - - Fe concentration in mg/g dry weight or Co concentration in μg/g dry weight

In all the statistical data reported herein, no significant effect was observed that could be attributed to the tracer. The critical ratio fertiliser P uptake/soil P uptake was also unchanged at either 2 or 4 μc P^{32} per pot. It is concluded therefore that the low levels of radioactive phosphate used did not affect the results of the nutritional experiment.

4. *Effect of Cobalt and Phosphate on Redistribution of Dry Weights and Phosphate between Tops and Roots*

The effect of cobalt and fertiliser phosphate treatments on the redistribution of dry weights, total and fertiliser phosphate contents

between 'tops' and 'roots' has also been analysed. The data at the 1st harvest show that with increasing cobalt level the reduction in dry matter, total and fertiliser phosphate is somewhat less marked in the roots than in the tops. There is no effect due to cobalt at the 2nd harvest. Increasing the fertiliser phosphate level applied to the plants results in a reverse effect at both harvests for all three functions. In each case at the higher fertiliser level, a redistribution in favour of tops has occurred.

DISCUSSION

The aim of the present investigation was to examine the effects of cobalt at toxicity levels in tomato on (*a*) the production of iron deficiency, and (*b*) phosphate contents in roots and tops respectively. Three rates of cobalt, at two fertiliser levels, each labelled with three P^{32} rates were studied. Iron deficiency symptoms developed in the high Co treatments in the young leaves within a few days of applying the metal and were more marked at the lower fertiliser phosphate level. Chlorosis was followed by direct metal injury effects, namely necrosis and death of the leaf and petiole tissues. These results are in agreement with those of Somers and Shive[19]), Hewitt[6]), Millikan[13]) and Nicholas[16]).

Previous work has shown that metal induced — (Millikan[13]), Wallace and Hewitt[21])) and lime induced — (Wallace and Mann[22]), Lindner and Harley[10])) — iron deficiency seldom result from a shortage of total iron (expressed as μg/g in dry matter), in the chlorotic leaves. In some instances the chlorotic leaves contain a higher concentration of total iron than green leaves, thus indicating a possible inactivation of iron in the tissues. This phenomenon has led Oserkowsky[17]), Lindner and Harley[10]) and Nicholas[16]) to fractionate the total iron in leaves and thus determine the 'active' iron in the tissues. Somers and Shive[19]) suggested a mechanism involving the oxidation reduction potentials of the simple inorganic cations for the oxidation of ferrous iron with the formation of a ferric phosphate organic and complex. The hypothesis was shown to be unsound by Leeper[9]) and experimental evidence with a number of heavy metals confirming its inadequacy was obtained by Hewitt[5]). The injurious effects of Co, reported in this paper were alleviated by the higher phosphate treatment. The application of Co depressed all the

various P fractions in the plants other than soil P at the 2nd harvest and Co_4 level only. It appears therefore that an adequate fertiliser P level in the tissues is important to overcome the metal injury.

In the present experiments the fertiliser phosphate increased dry weights of roots and tops whereas the higher Co treatments depressed them. The total P and fertiliser P contents were decreased with increased Co treatment, thus showing similar trends to those of dry weights. When the values were expressed as a concentration in dry matter these effects were still significant, so this decrease was independent of reduction of dry weight by Co. At the 2nd sampling there was a greater amount of soil P in the plant treated with a higher level of fertiliser phosphate, probably due to growth stimulation by the fertiliser. On a dry matter concentration basis the soil P content of the plants was greater at the higher cobalt levels, an effect which was opposite to that of cobalt on fertiliser phosphate concentration in dry matter, thus demonstrating that on a dry matter concentration basis the two fractions behaved differently at the 2nd harvest.

Biddulph and Woodbridge [1]) showed that ferric phosphate is precipitated in or on the roots of bean plants grown in water culture and that this retarded the flow of iron and phosphate to the aerial parts of the plants. Sideris, Young and Krauss [18]) also demonstrated a fixation of iron in the roots of *Ananas comosus* when phosphate was increased in the liquid medium. The effect of cobalt in changing the ratio of dry matter and phosphate in favour of the roots of chlorotic plants (section 2, 4) is of interest, though at each cobalt level, increasing fertiliser phosphate produced a change of dry matter and phosphate in favour of plant tops.

The effect of high fertiliser phosphate levels in producing symptoms of iron deficiency is well known and has been observed by many workers. In the presence of cobalt however the injurious effects of cobalt were less marked at the higher fertiliser phosphate level (Plate II), which indicated that one of the main effects concerned was a phosphate-cobalt interaction. An examination, now in progress, of the distribution of P in young and old leaves of normal and chlorotic plants may elucidate further the role of P in metal induced iron chlorosis. When Fe was expressed in μg per g dry weight, there was no significant difference due to cobalt, so that the uptake of Fe (p.p.m.) paralleled a decrease in dry weights. The cobalt contents of the plants reflected Co treatments.

Millikan [11]) found that molybdenum may alleviate the effects of toxic concentrations of heavy metals, including cobalt, in flax grown in water culture experiments.

In the experiment in this paper in the P_2^{31}, P_4^{32}, Co_4 treatment, Mo at 2 rates depressed still further the dry weights and total P, fertiliser P in roots and tops, and soil P in the whole plant and was without effect on the total iron content (as p.p.m. dry matter) of the plants. It is unlikely, under these conditions, that Mo would be of use as an antidote to cobalt injury in tomato. This confirms the findings of Warington [23]), Hewitt [7]), Nicholas [16]), in water, sand and soil culture respectively.

Two low activities of P^{32}, 2 and 4 μc per pot were used in the experiments. The tracer did not significantly affect dry weights and phosphate, iron and cobalt contents of roots and tops. In particular the critical ratio fertiliser uptake/soil P uptake was not affected so that it is reasonable to assume that the tracer did not affect the nutrition of the plants during the experiments.

SUMMARY

1. The effects of soil applications of cobalt on tomato have been studied in a factorial pot experiment. Three rates of cobalt, 0, 1 and 4 milliequivalents respectively per pot, and two rates of fertiliser phosphate equivalent to 500 and 1000 lbs. superphospate per acre were applied to the soil. Each rate of fertiliser phosphate was labelled with three levels of P^{32} viz. 0, 2 and 4 μc per pot.

2. At both harvests, dry weights, total P and fertiliser P contents in tops and roots were depressed at the highest Co level and increased at the higher fertiliser P treatment. These effects were observed when P was calculated as total mg or expressed as a concentration in dry matter. This indicates that the effects of cobalt and fertiliser P on total P and fertiliser P were independent of dry weight yields. At the 2nd harvest however the Co_4 treatment increased the soil P fraction in the plants so that the metal affects the fertiliser and soil P differently at this stage.

3. The injurious effects of cobalt on the plants were much less marked at the higher fertiliser phosphate level, indicating a probable phosphate-cobalt interaction.

4. Although symptoms of iron deficiency were readily produced at the highest Co level, the total Fe expressed in μg per g dry weight (p.p.m.) was not significantly affected.

5. The addition of sodium molybdate at 5 and 10 milliequivalents per pot respectively accentuated the injurious effects of cobalt, decreasing the dry

weight yields and total P, fertiliser P and soil P uptake, but was without effect on the iron status of the tops and roots.

6. P^{32} at 2 and 4 μc per pot did not appear to effect any changes in the nutrition of the plants. It is therefore unlikely that the tracer affected the plants in these experiments.

ACKNOWLEDGMENTS

We are grateful to Mr. D. J. Fisher for assistance with the analytical work, and to Messrs. H. M. Webber, M. A. Wright and J. Sims, who helped us to harvest the plants. The last named also assisted with the radio assay.

Received May 23, 1953.

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Lime-induced manganese deficiency in Lady Sylvia rose.
Left : chlorotic leaf showing netted appearance and reduced size.
Right : Normal leaf.

Plant and Soil V, no 1 October 1953

LIME-INDUCED MANGANESE DEFICIENCY IN GLASSHOUSE ROSES

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A chlorosis of glasshouse roses has occurred sporadically for some years. It does not appear to have persisted for any length of time in some nurseries nor has it always reappeared in the same places in successive years. In one case a complete house was seriously affected one year, yet the following year all the same trees made normal growth and produced satisfactory foliage. During the years 1950 and 1951, however, the incidence of the disorder was much more pronounced and it was found in appreciable amount on six different nurseries. It seems to be generally accepted among growers that it is due to undesirable soil conditions and the view has been frequently expressed that growing tomatoes instead of roses during World War II has been an important factor.

The chlorosis starts as a loss of colour between the veins. At first interveinal areas are pale green but later these become yellow. Meanwhile the veins, even the finest of them, retain their original green colour so that the leaf as a whole has a characteristic delicate netted appearance. The leaf never becomes wholly yellow; there is usually no necrosis nor is there any extensive leaf fall. In general the size of the leaf is reduced as will be seen from the plate. Frequently the condition develops as the leaf grows but later disappears as the flower bud swells and opens. In other cases, however, the condition persists and worsens so that the flower stems become weak and the blooms have little value. In one such case growth generally was so weak that comparatively young trees were dug up and discarded. The distribution of chlorotic trees is erratic. On some nurseries whole houses are more or less affected. On others, parts only of houses show the disorder whilst in others there are continuous areas ex-

tending over parts of two or even three adjacent houses in a block. The evidence concerning susceptibility is somewhat conflicting and a variety which may be seriously affected on one nursery has made normal growth on another. Varieties which have been affected include Lady Sylvia, Autumn, Talisman, Roselandia and Richmond.

The symptoms correspond with those described by Laurie and Kiplinger[3]). Presumably these effects were induced artificially as we have been unable to find any record in the literature of the occurrence of this disorder under commercial conditions. While the work was in progress, however, we were informed by Miss Ir. C. Dröge[1]) (private communication) that this chlorosis occurs in commercial glasshouses in Holland. There it is sometimes attributed to a deficiency of iron.

Quantitative tests on normal and chlorotic leaves of some of the affected varieties indicated high calcium and low manganese in the affected leaves suggesting a calcium-induced manganese deficiency.

Quantitative examination of two sets of leaves of the variety Roselandia gave the following results:

TABLE I

Analysis of Roselandia leaves						
	N%	Ca%	Mg%	P%	Fe p.p.m.	Mn p.p.m.
Normal leaves	3.00	1.52	0.55	0.26	365	90
Chlorotic leaves	1.78	3.34	0.62	0.16	297	62

These values suggest that deficiencies of nitrogen, phosphorus, iron or manganese respectively or a deficiency of all are operating and that an excessive uptake of calcium is contributory. That the deficiency is of manganese has been demonstrated by growing some of the chlorotic Roselandia trees in sand culture. Only those from which manganese was withheld continued to show the characteristic chlorosis.

It is concluded therefore that the disorder is due to manganese deficiency and in view of the fact that all the soils where it has occurred are alkaline and contain free calcium carbonate, that it is lime-induced.

Among the corrective measures which were tried in preliminary experiments were:

1. Application of solid manganese sulphate to the beds at the rate of 2 ozs. per sq. yard. During the ordinary cultural operation this was

watered in. The treatment was quite ineffective. This was not surprising in view of the fact that the soil contained free carbonate and it was not considered advisable to use a higher rate of application of manganese lest a toxic concentration be reached.

2. Leaf and stem injections with dilute manganese sulphate solutions. These were without effect.

3. Foliage spraying with various strengths up to 0.5% w/w of manganese sulphate plus either Estol H or sulphonated lorol as wetters. At the lower concentrations used there was no apparent effect. Nearer to and at the maximum concentration, noticeable injury was done to both foliage and the flower buds.

4. Treatment of the beds, in which the trees grew, with flowers of sulphur. This provided a spectacular success on one nursery the first time it was tried. This treatment is, of course, well-known and is commonly used to reduce the alkalinity of soils and so correct lime-induced deficiencies. In this particular trial the sulphur was pricked into the top three or four inches of the soil when the trees were dormant and the beds were bare of mulch, trimmings, etc. From laboratory tests it appeared that 4 ozs. per sq. yard should be adequate and this was the dressing that was used. It was appreciated that some root injury might follow the pricking in but it was hoped that it would be more than compensated for by correction of the disorder. The hope was justified in that during the whole of the following season the trees in the treated beds remained a healthy green and were much stronger than the trees in the untreated beds.

During the following winter the sulphur treatment was carried out on a number of nurseries. The final result was the same, that is to say normal green growth was the rule where sulphur had been applied. In all cases the rate of application was 4 ozs. per sq. yard and it was made when the trees were dormant. All the varieties mentioned were treated in these trials and some differences in varietal response were observed. Lady Sylvia and Roselandia responded as in the first year *i.e.* normal colour with no chlorosis was general. Growth generally was sturdier than in the untreated trees and in one nursery there was a marked increase in the formation of new shoots low down on the trees. The varieties Autumn and Talisman however showed slight retardation of the rate of growth in the early season, but this effect was only transient and eventually the trees were definitely superior to those in untreated plots.

Richmond merits special mention. The trees in the treated beds suffered a decided retardation in development during the first few weeks of the growing season. This variety is not a particularly strong one ordinarily. At the relevant times, however, growth was weaker than usual and despite the fact that the foliage was a good colour the new growth was stunted. Later the rate of growth accelerated and by mid-season was quite satisfactory. By the autumn the treated trees were in considerably better condition than the untreated ones. Samples of foliage from both lots of trees of this variety taken in May gave the following analytical values:

TABLE II

Analysis of Richmond leaves							
	N%	Ca%	Mg%	P%	K%	Fe p.p.m.	Mn p.p.m.
Sulphur treated	2.37	1.28	0.36	0.26	1.79	87	106.5
Untreated	2.28	1.29	0.34	0.20	1.84	71	23.5

These results show that the most pronounced effect of the treatment is the increased uptake of manganese.

The retardation of growth may be due to one of several causes. It may be related to the fact that Richmond has a comparatively shallow rooting habit. It is grafted on *Rosa manetti* whereas the other varieties are on *R. canina*. The effects observed may be due to sulphur toxicity or the formation of some injurious compound during its oxidation. Alternatively it may be due to manganese toxicity.

Another striking feature of the analytical results is the difference in the iron content of Richmond and Roselandia. This may be varietal but it may also be due to the fact that the two varieties were grown on different nurseries some miles away from one another.

Since the foregoing trials were carried out sulphur applications without any pricking in have been made. It appears that results are the same so that the risk of mechanical root damage can be obviated.

While the nursery trials were in progress laboratory tests were carried out on the rate of disappearance of sulphur from some of these soils. Preliminary experiments indicated that 0.2% sulphur mixed intimately with soil would decompose fairly quickly and reduce pH values markedly. In one experiment a rose soil with an initial pH value of 7.38 was mixed with 0.2% by weight of sulphur and incubated at 23.5°C. The pH fell to 6.05 in 5 weeks and almost

70 per cent of the sulphur had been converted into sulphate. It was on this basis that it was decided to use a dressing of 4 ozs. per sq. yard. Temperature must have a pronounced effect on these changes and under no ordinary conditions would a rose soil attain a temper-

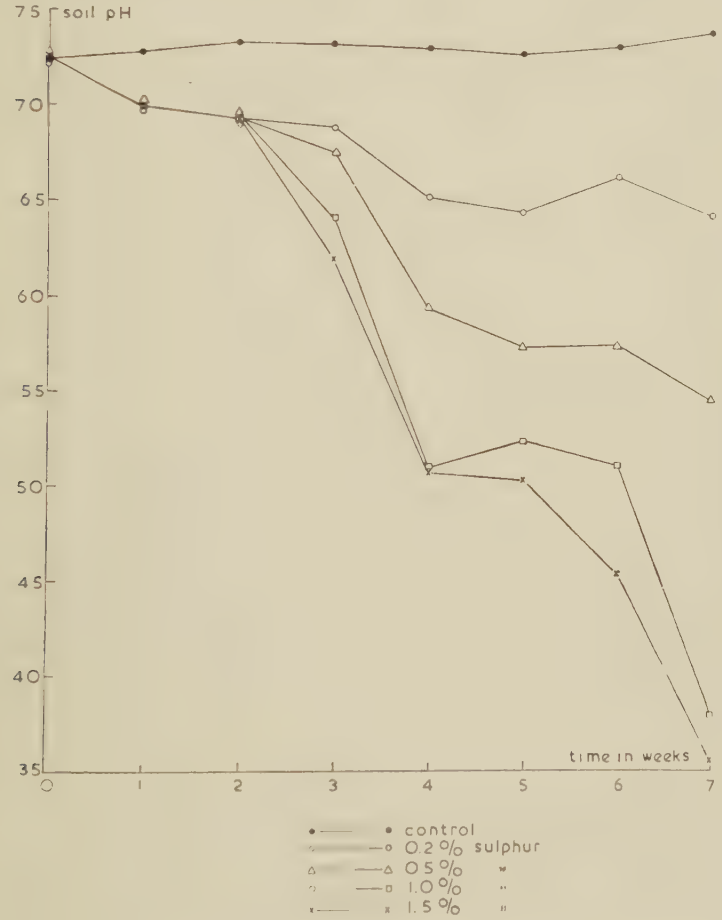

Fig. 1. The effect of various concentrations of sulphur on soil pH.

ature of 23.5°C. The result would be that the changes observed would take place much more slowly than those recorded during the incubation.

It would seem from the foregoing that 4 ozs. per sq. yard is near the maximum amount of sulphur necessary to bring about the

desired effects. As a matter of interest it was decided to determine what effects heavier dressings would produce during incubation. A composite sample of soil from an affected glasshouse with an initial pH of 7.33 was divided into subsamples which were mixed intimately with different amounts of sulphur and incubated at 23.5°C for seven weeks. The results are depicted in fig. 1 and the final pH values are as follows:

	Final pH values
Original pH	7.33
Soil + 0.2% sulphur	6.44
,, + 0.5% ,,	5.47
,, + 1.0% ,,	3.80
,, + 1.5% ,,	3.55

Reference to the curves shows that reduction in pH values is not complete except possibly at the 0.2 per cent level and it is likely that at the higher concentrations of sulphur further incubation would have produced still lower values. It is unlikely that there would be any advantage in this and it is doubtful whether reduction to below pH 6.0 is necessary or even desirable. Garey and Barber [2]) suggest the oxidation of sulphur is the important factor in raising the level of available manganese and low pH is only effective in producing conditions favourable for the existance of the available form. This brings up the question as to whether smaller dressings than that used in the trials would be sufficient for the purpose in view. This is a matter for further trial.

SUMMARY

A characteristic chlorosis of glasshouse roses is shown to be due to a lime-induced manganese deficiency. The disorder has been corrected by an application of elementary sulphur to the borders.

Received June 2, 1953.

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Plant and Soil V, no 1 October 1953

THE RELATIVE PROPORTIONS OF THE CHLOROPLAST PIGMENTS AS INFLUENCED BY DIFFERENT LEVELS OF IRON AND POTASSIUM SUPPLY

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INTRODUCTION

The visual study of various mineral deficiency symptoms involves a description of leaf colour. Many of these symptoms cause a marked chlorosis or yellowing of the lamina, as in iron deficiency chlorosis. It is difficult to judge from a study of the literature whether this yellowing is due to a general reduction in pigment content, with no change in the relative proportions of the individual pigments, or whether the proportion of chlorophyll to the carotenoid pigments is altered. In addition, it was found in unpublished investigations that in instances of mild iron deficiency chlorosis, in which the young leaves appear paler than the old, the chlorophyll content of the young laminae was greater than that of the old when expressed on a dry weight basis.

To study these problems and to provide a firmer background to the description of leaf symptoms rapid methods were devised or adapted for the assessment of leaf pigments. A preliminary study was made of the variation in pigment content of potato laminae maintained under varying conditions of iron status.

Willstätter and Stoll[12]) and Griffith, Valleau and Jeffrey[5]) observed that the ratio of green to yellow pigments in green leaves was remarkably constant. Hinkle and Eisenmenger[7]) also found that the ratios were the same in chlorotic magnesium-deficient and green leaves.

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A similar constancy was observed by Mackinney[9]) for normal and iron deficient leaves. Oserkowsky[10]) however, found that chlorotic iron deficient leaves had different ratios of chlorophyll to yellow pigments than green leaves. Both Oserkowsky and Mackinney reported a close correlation between chlorophyll and the carotenoid pigments.

METHODS

Treatments

Iron and potassium were applied at three different levels chosen to represent deficiency, normal and luxury consumption. These were in milliequivalents per litre:

$$Fe_1 = 0.0016; \quad Fe_2 = 0.05; \quad Fe_3 = 1.00;$$
$$K_1 = 0.6; \quad K_2 = 2.4; \quad K_3 = 9.6.$$

The phopshorus supply was kept constant at the high level of 8.1 meq. of phosphate per litre necessary to produce the desired effects of iron and potassium deficiency in the various nutrient combinations employed. The general composition of the nutrients used, their preparation and a general description of the pot sand culture technique are given by Hewitt[6]). The experiment was laid out in two blocks; each plot comprised three pots. Potato tubers, variety Majestic, were planted 26/6/51.

Sampling

Young and old laminae were sampled for analysis; the 'young' being removed from the second or third node from the tip of the shoot and the 'old' from the midstem area, i.e. regions exhibiting iron and potassium deficiency respectively. Approximately 10 g of lamina were sampled from each treatment and the midribs excised, prior to discs of known area being punched out with a cork borer.

The discs, each 2.064 sq. cm in area were well mixed and apportioned in the following fashion and stored in corked specimen tubes: 12 discs each for dry weight and chlorophyll determinations and 24 discs for carotene and 'xanthophyll' determination. 12 discs roughly corresponded to 0.5 g fresh weight. Discs used for dry weight determinations were weighed fresh, dried in a well aerated oven at 80°C and reweighed after 24 hours; those used for pigment estimation were stored in solid carbon dioxide until needed for extraction.

Extraction and Estimation of Pigments

Samples to be extracted were thawed quickly, transferred to a pyrex glass tube and a pinch of carborundum (No. 90) added together with 1–2 ml of extractant. A mechanically driven pyrex pestle was inserted into the tube and rotated until the plant material was completely macerated; usually this stage was reached in 20 seconds — before the tube warmed appreciably. The chlorophyll samples were extracted with 85% acetone in the presence of about 5 mg of calcium carbonate, using similar conditions to those employed by Petering, Wolman and Hibbard [11]. The macerate was washed into a Buchner funnel, filtered through a Whatman No. 42 filter paper and repeatedly washed until no more green colour remained in the residue. The filtrate was made up to 50 ml with 85% acetone and 'Spekkered', using red filters. The calibration curve was obtained by adapting the methods described by Comar [2], Comar and Zscheile [3] and Comar, Benne and Butevn [4]. The critical measurements of the % transmittance of a chlorophyll in ether solution at 6425 Å and 6600 Å were carried out at the National Physical Laboratory. The values presented here are those for total chlorophyll content.

The 24 discs used for the carotenoid estimation were macerated with a solvent consisting of equal parts of acetone and light petroleum (40–60°C) and containing 0.1% of quinol. When the maceration was complete the mixture was washed with more solvent and finally filtered; it was arranged that the total volume of the filtrate should not exceed 50 ml. The extract was washed free of acetone with approximately 1 litre of tap water in the continuous washing apparatus described by the Analytical Methods Committee of the Analyst [1], who also described the solvent used and the bone meal separation procedure referred to below. The light petroleum layer which remained after washing was made up to 50 ml – and of this 25 ml were used for the carotene estimation. This aliquot was passed through a bone meal column; the carotene eluted directly whilst the other pigments remained at the top of the column. The eluate was made up to 50 ml with light petroleum and 'Spekkered' using blue filters. The carotene content was calculated by reference to a calibration curve prepared with β-carotene.

The remaining 25 ml of the original light petroleum extract was transferred to a separating funnel and 50 ml of saturated potassium

hydroxide and 25 ml of a 1 : 1 mixture of methanol and propanol added. (This procedure was suggested by Dr V. H. Booth of the Agricultural Research Council staff). The funnel was shaken vigorously for one minute and then allowed to stand for twenty minutes. The lower aqueous layer containing the saponified chlorophyll pigments was run off and 30–40 ml of light petroleum added followed by

TABLE I

Chlorophyll content (expressed on area, fresh and dr

Treatment		September 11th								
		mg Chlorophyll			Fresh weight of 24.77 sq. cm (g)	Dry weight of 24.77 sq. cm (g)	% Dry matter	mg Chlorophyll		
		per 100 sq. cm	per gram fresh weight	per gram dry weight				per 100 sq. cm	per gram fresh weight	per gram dry weight
Block 1										
Fe_1K_1	Young	0.55	0.29	3.78	0.475	0.036	7.58	1.14	0.53	5.79
	Old	1.51	0.67	4.54	0.508	0.074	14.49	1,61	0.86	8.97
Fe_1K_2	Young	2.66	1.54	15.00	0.430	0,044	10.23	—	—	—
	Old	2.50	1.01	11.70	0.614	0.053	8.70	—	—	—
Fe_1K_3	Young	2.72	1.43	14.36	0.473	0.047	9.94	2.94	1.39	12.26
	Old	2.97	1.18	12.25	0.621	0.060	9.62	3.41	1.46	14.29
Fe_2K_2	Young	2.60	1.36	13.72	0.475	0.047	9.90			
	Old	3.01	1.30	12.02	0.572	0.062	10.78			—
Fe_3K_3	Young	3.13	1.60	19.38	0.485	0.040	8.25	—	—	—
	Old	3.13	1.42	12.30	0.647	0.063	9.68	—	—	—
Block 2										
Fe_1K_1	Young	0.45	0.22	2.16	0.510	0.052	10.12	0.80	0.39	4.37
	Old	1.98	0.84	6.90	0.581	0.071	12.27	1.35	0.68	6.02
Fe_1K_2	Young	3.25	1.73	14.91	0.465	0.054	11.61	—	—	—
	Old	3.01	1.13	11.82	0.659	0.063	9.59			—
Fe_1K_3	Young	2.97	1.54	12.90	0.476	0.057	11.97	3.02	1.54	12.99
	Old	2.89	1.08	11.53	0.665	0.062	9.35	2.78	1.17	11.75
Fe_2K_2	Young	3.13	1.64	14.35	0.471	0.054	11.46			—
	Old	3.13	1.17	11.07	0.661	0.070	10.54	—	—	—
Fe_3K_3	Young	2.68	1.35	13.57	0.494	0.049	9.92			—
	Old	2.99	1.38	14.86	0.537	0.050	9.27	—	—	—

a similar volume of distilled water. The light petroleum layer was scrubbed in the continuous washing apparatus, *loc. cit.*, with 1.5 l of distilled water to remove all traces of alcohol, run off into a volumetric flask, made up to a volume of 50 ml and 'Spekkered' using blue filters. The drum readings were referred to the carotene calibration curve. This procedure gave the approximate total content of

weight basis) and dry matter content of young and old leaves

September 24th			October 1st			October 3rd					
						mg Chlorophyll					
Fresh weight of 24.77 sc. cm (g)	Dry weight of 24.77 sq. cm (g)	% Dry Matter	Fresh weight of 24.77 sq. cm (g)	Dry weight of 24.77 sq. cm (g)	% Dry Matter	per 100 sq. cm	per gram fresh weight	per gram dry weight	Fresh weight of 24.77 sq. cm (g)	Dry weight of 24.77 sq. cm (g)	% Dry Matter
0.534	0.049	9.16	0.455	0.040	8.69	1.11	0.52	7.91	0.533	0,035	6.55
0.462	0.045	9.64	0.468	0.054	11.49	2.66	1.29	12.89	0.511	0.051	10.04
			0.515	0.066	12.71	2.44	1.10	15.90	0.551	0.038	6.91
—	—	—	0.688	0.070	10.18	2.86	1.13	11.68	0.626	0.061	9.70
0.526	0.060	11.30	0.542	0.060	11.15	2.61	0.98	10.85	0.660	0.060	9.05
0.578	0.059	10.24	0.750	0.069	9.24	2.98	1.14	13.75	0.647	0.054	8.31
—	—	—	0.473	0.067	14.09	3.30	1.65	15.52	0.496	0.053	10.63
—	—	—	0.605	0.075	12.44	3.66	1.59	14.11	0.570	0.064	11.28
—	—	—	0.561	0,076	13.51	2.92	1.20	11.32	0.601	0.064	10.64
—	—	—	0.641	0.057	8.88	2.89	1.05	12.06	0.681	0.059	8.73
0.512	0.046	8.90	0.465	0.042	8.94	1.07	0.51	6.49	0.521	0.041	7.86
0.494	0.056	11.26	0.420	0.060	14.39	1.48	0.76	7.22	0.481	0.051	10.56
	—	—	0.560	0.060	10.65	2.82	1.43	17.18	0.490	0.041	8.30
	—	—	0.629	0.067	10.71	2.44	0.81	8.88	0.749	0.068	9.08
0.484	0.058	11.89	0.609	0.060	8.78	2.60	1.03	11.01	0.624	0.059	9.39
0.590	0.059	9.95	0.908	0.070	7.76	2.65	0.85	10.61	0.775	0.062	7.99
	—	—	0.495	0.062	12.52	3.02	1.56	13.46	0.482	0.056	11.56
			0.617	0.068	11.08	3.12	1.19	10.00	0.652	0.078	11.89
	—	—	0.599	0.056	8.23	2.72	1.21	13.43	0.558	0.050	9.00
			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	11.1	0.434	[illegible]	13.27

the yellow pigments present and subtraction of the carotene values from this total gave an estimate of the 'xanthophyll' present.

RESULTS

Iron deficiency chlorosis and potassium deficiency symptoms appeared one month after planting. Iron deficiency was marked in the Fe_1K_1 treatment and was also present to a slight extent in Fe_1K_2. Potassium deficiency was severe in the Fe_1K_1 treatment and occurred to a lesser extent in the Fe_1K_2 and Fe_2K_2 treatments. For other treatments the plants were generally healthy, vigorous and normal green in colour.

The old laminae contained more dry matter per unit area than the young — Table I; this difference was especially great in the Fe_1K_1-plants, i.e. those showing chlorosis. The visual method of description was subjective and based on leaf area whereas the chemical method was based on weight. Consequently in instances of mild chlorosis, if the results were calculated on a dry weight basis and the young were found to contain more chlorophyll than the old laminae the discrepancy could be explained in terms of the different area/weight ratios.

The chlorotic plants were the only ones which consistently showed a higher percentage dry matter in the old than in the young laminae. Table I shows that the disparity between the chlorophyll content of the young chlorotic leaves and the old pale green ones was best illustrated on the area basis, followed by fresh weight and then dry weight. It was therefore decided to present the pigment analysis on an area basis.

The application of potassium up to the K_2 level increased the chlorophyll, carotene and xanthophyll contents of both young and old laminae — Table II; there was little further increase at the K_3 level. Iron application increased the chlorophyll content, more so at the K_2 than at the K_3 level; iron, to a lesser extent, also increased the carotene and xanthophyll contents. The ratio values of chlorophyll/carotene in the young laminae increased with the severity of the chlorosis except for the later samplings — Table III. This trend was not so obvious in the old laminae and was even reversed in the later samplings. When the September ratio values of chlorophyll/carotene in the young laminae area considered separately the differ-

TABLE II

Micrograms of pigment present in 12 lamina discs (24.77 sq. cm). Mean values

Treatment	Young					Old		
	Sept. 6th	Sept. 11th	Sept. 24th	Oct. 3rd	Oct. 22nd	Sept. 11th	Sept. 24th	Oct. 3rd
	Chlorophyll							
Fe_1K_1	228	125	241	271	140	432	368	348
Fe_1K_2	711	732	—	652	—	632	—	623
Fe_1K_3	752	705	739	647	612	725	768	698
Fe_2K_2	817	710	—	807	—	760	—	841
Fe_3K_3	808	720	—	699	—	758	—	728
	Carotene							
Fe_1K_1	10.5	5.5	14.0	13.5	11.7	26.5	21.0	28.0
Fe_1K_2	48.8	56.2	—	42.0	—	46.0	—	40.7
Fe_1K_3	52.2	50.8	48.5	50.5	36.9	50.2	48.5	40.8
Fe_2K_2	56.0	57.0	—	50.1	—	52.0	—	49.1
Fe_3K_3	58.2	60.5	—	46.8	—	49.2	—	46.0
	Xanthophyll							
Fe_1K_1			49.0	42.4	31.6		54.5	58.2
Fe_1K_2			—	72.5	—		—	76.6
Fe_1K_3			74.0	68.0	65.8		87.5	80.2
Fe_2K_2			—	75.5	—		—	75.2
Fe_3K_3			—	88.1	—		—	75.5

ences between the Fe_1K_1 and Fe_1K_3 treatments are significant at the 2% level. The ratio of chlorophyll xanthophyll was smaller in the chlorotic plants than in the green plants — sometimes being less than half the value in the young laminae Table III. The differences between the chlorophyll xanthophyll values of the Fe_1K_1 and Fe_1K_3 treatments were significant at the 1% level for both young and old leaves. The ratio of carotene xanthophyll — Table III, was much lower in chlorotic (Fe_1K_1) than in green laminae (Fe_1K_3) - this difference was significant at the 0.1% level in the young but was not always consistent in the old laminae.

Based on the chlorophyll and carotene contents of 64 leaf samples and on the xanthophyll contents of 34 of these samples the following regression equations were evaluated:

I. Regression of Xanthophyll (x) on Chlorophyll (z)
II. Regression of Carotene (y) on Chlorophyll (z)
III. Regression of Xanthophyll (x) on Carotene (y)

All three regressions were found highly significant and the equations were:

TABLE III

Ratios of Chlorophyll to Carotene (Q/C); Chlorophyll to Xanthophyll (Q/X); and Carotene to Xanthophyll (C/X)

Treatment	Block 1 Young								Block 2 Young									
	Sept. 6th	Sept. 11th	Sept. 24th			Oct. 3rd			Sept. 6th	Sept. 11th	Sept. 24th			Oct. 3rd			Oct. 22 *)	
	Q/C	Q/C	Q.C	Q.X	C/X	Q.C	Q.X	C/X	Q/C	Q/C	Q/C	Q/X	C/X	Q.C	Q/X	C/X	Q/C	Q/X	C/X
Fe_1K_1	20.00	27.23	16.64	5.00	0.30	15.33	6.11	0.40	23.18	18.78	18.09	4.85	0.27	29.56	6.71	0.23	12.08	4.50	0.37
Fe_1K_2	13.64	12.56	—	—	—	14.29	8.01	0.56	15.58	13.42	—	—	—	16.81	10.07	0.60	—	—	—
Fe_1K_3	14.77	14.52	15.53	10.43	0.67	8.15	9.82	0,83	14.12	13.37	14.96	9.60	0.64	14.03	9.21	0.66	16.59	9.35	0.56
Fe_2K_2	14.51	11.52	—	—	—	14.82	10.65	0.72	15.25	13.45	—	—	—	16.66	10.73	0.58	—	—	—
Fe_3K_3	14.16	11.74	—	—	—	15.88	9.39	0.59	13.57	11.81	—	—	—	14.05	6.74	0.48	—	—	—
	Block 1 Old								Block 2 Old									
Fe_1K_1	—	17.44	16.00	6.78	0.42	11.78	4.52	0.38	—	15.56	19.71	6.70	0.34	13.11	8.07	0.65	—	—	—
Fe_1K_2	—	15.12	—	—	—	16.46	8.36	0.51	—	14.62	—	—	—	14.24	7.92	0.56	—	—	—
Fe_1K_3	—	14.85	18.00	8.72	0.48	16.23	8.12	0.50	—	14.03	13.80	8.85	0.64	18.25	9.62	0.52	—	—	—
Fe_2K_2	—	14.76	—	—	—	16.44	12.04	0.73	—	14.48	—	—	—	18.02	10.33	0.57	—	—	—
Fe_3K_3	—	15.51	—	—	—	15.58	9.37	0.60	—	15.26	—	—	—	16.04	9.91	0.62	—	—	—

*) Estimations carried out on material sampled from both blocks and then bulked.

	a (constant term)	*b* (slope)
I. Xanthophyll on Chlorophyll:	$x = 28.3$	$+ 0.0671\ Z$
	$(\pm\ 4.168)$	$(\pm\ 0.0067)$
II. Carotene on Chlorophyll:	$y = -1.58$	$+ 0.0698\ Z$
	$(\pm\ 1.870)$	$(\pm\ 0.0029)$
III. Xanthophyll on Carotene	$x = 28.7$	$+ 1.038\ Y$
	$(\pm\ 4.277)$	$(\pm\ 0.1102)$

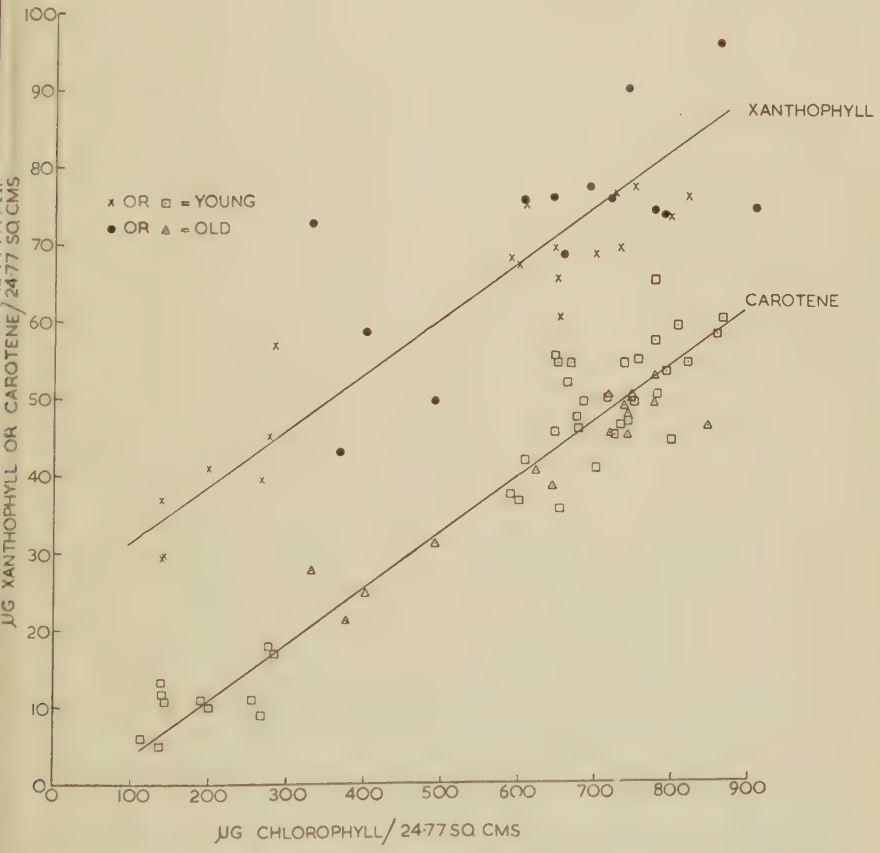

Fig. 1. The relationship of the Carotene and Xanthophyll to the Chlorophyll content of the lamina.

No attempt was made to differentiate between values obtained for young and old laminae as it was thought the data available did not warrant any conclusions based on such a differentiation. The slopes

of equations I and II did not differ significantly (difference =0.0027 ± 0.0075), but a marked difference was established between their constant terms (difference = 29.88 ± 4.57). Hence the regressions of xanthophyll on chlorophyll and of carotene on chlorophyll can be regarded as two parallel lines (see Fig. 1).

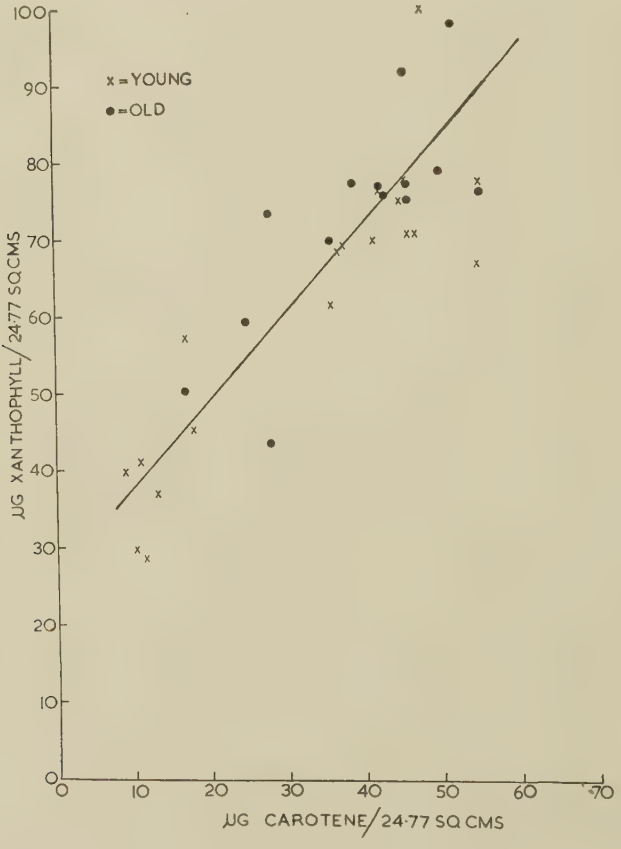

Fig. 2. The relationship of the Carotene to the Xanthophyll content of the lamina.

The constant term in equation II is —1.58 (s.e. ± 1.87)and hence did not differ significantly from zero; thus the limits of error of the carotene/chlorophyll regression were such that the line may in fact pass through zero (see Fig. 1). Equation III and Fig. 2 however indicate that plants completely deficient in carotene might still contain xanthophyll.

The correlation and partial correlation coefficients were as follows:

Correlation coefficients	r	*d.f.*	*Probality P*
Xanthophyll and Chlorophyll	$r_{xz} = +0.86$	32	Highly significant ($P < .001$)
Xanthophyll and Carotene	$r_{xy} = +0.86$	32	Highly significant ($P < .001$)
Carotene and Chlorophyll.	$r_{yz} = +0.95$	32	Highly significant ($P < .001$)
Partial Correlation Coefficients			
Xanthophyll and Chlorophyll with Constant Carotene	$r_{xz.y} = +0.32$	31	Almost significant ($P < .1$)
Xanthophyll and Carotene with Constant Chlorophyll	$r_{xy.z} = +0.23$	31	Not significant ($P > .1$)
Carotene and Chlorophyll with Constant Xanthophyll	$r_{yz.x} = +0.80$	31	Highly significant ($P < .001$)

With constant carotene content the positive correlation between xanthophyll and chlorophyll was almost significant but much less marked than the association observed when the carotene level was ignored. The correlation between xanthophyll and carotene for constant chlorophyll content was not significant. It appeared therefore that the highly significant correlation observed when the chlorophyll content was ignored was due to the association between xanthophyll and chlorophyll and between carotene and chlorophyll.

The closest relation existed between carotene and chlorophyll. This highly significant positive association persisted irrespective of the xanthophyll level.

When the chlorophyll, carotene and xanthophyll contents were expressed as a proportion of the total pigment content, it was found that the proportion of xanthophyll was consistently higher in chlorotic than in the green laminae. The proportions of chlorophyll and carotene generally showed little variation under varying conditions of iron status but tended to increase slightly with potassium level. This table is not presented here.

DISCUSSION AND CONCLUSIONS

The method employed for xanthophyll estimation was tentative but served for comparative purposes.

There was an obvious and clear correlation between chlorophyll and carotene contents and a confirmation of the straight line relationship between the two pigments inferred by Mackinney [9]. It would appear from a figure presented by Oserkowsky [10] who determined carotene and xanthophyll together as the "yellow pigments" that leaves devoid of chlorophyll would probably contain the yellow pigments. It was impossible, as it was in this present work, to prove this point as none of the plants analysed were free of chlorophyll but it seems likely from a consideration of Figs. 1 and 2 and the statistical data presented that the pigments present were more likely to be in the xanthophyll class. In support of this it may be mentioned that experiments with etiolated barley plants have failed to demonstrate the presence of chlorophyll but revealed a preponderance of xanthophyll over carotene (Mackinney [9]).

It is apparent that both iron and potassium supplies affect pigment content. The addition of potassium at the deficiency level of iron (Fe_1) greatly increased the overall pigment content and ameliorated iron deficiency chlorosis; the effect of potassium, however, was not critical above the K_2 level. The effect of iron, although usually beneficial, was not marked when applied in conjunction with potassium at the K_3 level. The beneficial effect of potassium on iron utilization in the presence of limiting iron supplies has been discussed elsewhere (Jones [8]).

It was concluded that the relative proportions of chlorophyll, carotene and xanthophyll in the potato leaf do not remain constant under various conditions of iron status and that when iron was deficient the proportion of xanthophyll increased in relation to the chlorophyll and carotene.

It was also found that the expression of pigment content on an area basis gave results which were in better agreement with visual observations than those expressed on either fresh or dry weight basis.

ACKNOWLEDGEMENTS

We are grateful to the Agricultural Research Council for permission to publish this paper and to Mr. D. R. Westgarth of the

Rubber Research Institute of Malaya for his assistance in the statistical treatment of the data.

SUMMARY

1. Potato plants were grown in pot sand cultures at different levels of iron and potassium supply. Plants grown at the lowest level of iron developed iron deficiency chlorosis and potassium deficiency symptoms when maintained at the lowest level of potassium but not at the highest level.

2. Rapid procedures were developed for the extraction and estimation of chloroplast pigments from small samples of lamina — usually less than 1 g of fresh material.

3. The expression of pigment content on an area basis gave results which were in better agreement with visual observations than those expressed on either fresh or dry weight basis.

4. Both iron and potassium additions increased the chloroplast pigment content. Leaves exhibiting iron deficiency contained reduced quantities of all pigments per unit area of lamina.

5. A linear relationship existed between chlorophyll and carotene, chlorophyll and xanthophyll, and carotene and xanthophyll contents.

6. The results suggested that laminae completely deficient in chlorophyll would contain no carotene but might still contain xanthophyll.

7. The relative proportions of chlorophyll, carotene and xanthophyll do not remain constant under varying conditions of iron status. When iron is deficient the proportion of xanthophyll increased in relation to the chlorophyll and carotene contents.

Received July 4, 1953.

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